ICES WKARHOM2 REPORT 2015

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)

26–30 October 2015

Santa Cruz de Tenerife, Canary Islands, Spain

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Executive summary

The Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus, T. mediterraneus and T. picturatus*) (WKARHOM2) was held in Santa Cruz de Tenerife (Canary Islands, Spain) 26–30 October 2015. The meeting was co-chaired by Kélig Mahé (France) and Pierluigi Carbonara (Italy), and included twelve age readers from six institutes (five countries). The objectives of this workshop were to review, document and make recommendations on current methods of ageing Trachurus species.

This workshop was preceded by otolith exchanges in 2014, which were undertaken using WebGR. A total of 550 fish was sampled from the Atlantic Ocean (Eastern Channel, Celtic Sea, Bay of Biscay, Azores, Portuguese waters and Tenerife) and the Mediterranean Sea (Alboran Sea, South Adriatic Sea and Ligurian Sea). 19 readers from 8 countries (France, Germany, Spain, Ireland, Italy, Portugal, Netherlands and Norway) participated to this exchange. Among three *Trachurus* species, all data showed a very low precision with the percentage of agreement between 47 and 56% and a CV from 29 to 69%. The precision analysis showed the same level of precision between otolith sections and whole otoliths from the Ligurian Sea.

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, of some of the major difficulties in ageing otoliths of *Trachurus* species. The results of the comparison between different ageing techniques on the same set of fish, showed a bias intra-reader and so it is recommended to use only one ageing technique by each reader. Moreover, the precision of reading is the same between slices and whole otoliths and so there is not a best ageing technique for *T. trachurus*. The progress of reading showed a percentage of agreement close to 65% for *T. trachurus* and *Trachurus picturatus*. However, the percentage of agreement for *Trachurus mediterraneus* remained to 44.4% with a CV to 40. In fact, the next exchange must be target *Trachurus mediterraneus* as a priority. Finally, this group reached an agreement on a definition of an ageing guideline and a reference collection presented in this report and the aim is to employ these tools for all laboratories.

In memoriam - Alberto Murta

Alberto was an excellent scientist who was chair of the WKARHOM1 meeting in Lisbon in 2012.

1   Introduction

1.1   Terms of reference

The Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus, T. mediterreaneus and T. picturatus*) (WKAR-HOM2), chaired by Pierluigi Carbonara*, Italy, and Kélig Mahé*, France, was held in Santa Cruz de Tenerife (Canary Islands, Spain), 26–30 October 2015, to:

a) Review information on age determination, otolith exchanges and validation techniques on this species;

b) Estimate (relative) accuracy and precision of horse mackerel, Mediterranean horse mackerel and blue jack mackerel age determination in the main European fishing areas;

c) Identify causes of age determination error and provide species-specific guidelines for the improvement of precision and reduction of bias between readers and laboratories;

d) Update age reading protocols for each species;

e) Update otoliths reference collections and a database of otoliths images;

f) Discuss and propose the most appropriate validation methods of age and growth pattern of otolith, for every species and stocks;

g) Address the generic ToR's adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

WKARHOM2 will report by 16 November 2015 for the attention of SSGEIOM, WGBIOP, SCICOM and ACOM.

A pre-workshop exchange took place in 2014.

2   Life history of *Trachurus* species

2.1   Horse mackerel (*Trachurus trachurus*)

Horse mackerel is abundant and widespread in the tropical and temperate East Atlantic and Mediterranean, ranging from Norway to South Africa (Figure 2.1). Horse mackerel (or scad) *Trachurus* (Linnaeus, 1758) (Order: Perciformes, Family: Carangidae) is the only resident carangid species in the waters of northwestern Europe (Smith-Vaniz, 1986).

Figure 2.1: Distribution map of *Trachurus* (*In* Smith-Vaniz, 1986)

Horse mackerel is a southern species, reaching its northerly distribution limit in the northern North Sea. It is fished and landed mainly for human consumption, but the market in northern Europe is small and the larger part is exported. Horse mackerel form large shoals that occur in bottom waters and midwater during the day, whereas during the night they disperse and form a layer just off the seabed (Macer, 1977). The range of vertical migrations decreases during winter, when activity is lower (Nazarov, 1989). The species typically occupies shelf seas, down to 200 m, but specimens have been reported to depths of 500 m. Horse mackerel may grow to about 60 cm length, but are more common in the size range of 15-40 cm (Smith-Vaniz, 1986). They grow rapidly during the first years of life and much more slowly after age 3. They are reported to reach 40 years of age (Abunza *et al.*, 2003), although ageing methods are somewhat uncertain. Both growth and age at maturity fluctuate, possibly because of density-dependent responses to the extremely large fluctuations in year-class strength (ICES, 1991).

The otolith shape analysis (Stransky *et al.*, 2008) identified three subpopulations who could present different growth patterns: a northern, an Ibero-Mauritian and an eastern Mediterranean group (Figure 2.2).

Figure 2.2: Proposed stock separation of horse mackerel inferred from multivariate analysis of the Fourier descriptors and average shapes of the otoliths in the three groups of sampling areas, as revealed by multivariate analysis of the Fourier descriptors (*In* Stransky *et al.*, 2008).

2.2   Mediterranean horse mackerel (*Trachurus mediterraneus*)

Trachurus mediterraneus members of *Trachurus* genus (Perciformes, *Carangidae*) are widely distributed in the Mediterranean and Black Sea as well as east coast of the Atlantic from English Chanel to Morocco. This species is a schooling semi-pelagic species, most commonly found at about 20-250 m depth. Mediterranean horse mackerel prefers sandy bottoms and / or muddy when it moves near the bottom. The food habits of the species changes considerably along the lifespan. The juveniles feed primarily on planktonic *euphasiacea* and *mysidacea* while larger individuals prefer more fish as prey (Šantić 2003).

Regarding the reproduction season in the Table 2.1 are reported the spawning months for the Mediterranean basin. In general the reproduction season is included between the late spring and summer with some geographical differences. Indeed in the western part of the Mediterranean the spawning period is moved towards the late summer – autumn.

Table 2.1 – Spawning seasons from literature data are classified by author/year, method, area and GSA. The months with the presence of actively spawning females are blue coloured. GSI: gonad somatic index

Author	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Method	Area	GSA
Carrillo, 1978						■	■	■	■	■			maturity stage	Catalan Coast	6
Casaponsa, 1993						■	■	■	■	■			maturity stage - GSI	Catalan Coast	6
Sabatés, 1990							■	■	■	■			larvae	Catalan Coast	6
Nannini et al., 1997				■	■	■	■	■	■				maturity stage	Thyrrenian	9
Spedicato, 2013				■	■	■	■	■	■	■			maturity stage	Central-Southern Thyrrenian	10
Santic et al., 2006					■	■	■	■					GSI	Central Adriatic	17
Viette et al., 1997				■	■	■	■	■	■				maturity stage - GSI	Gulf of Trieste	17
Pesic et al. 2012				■	■	■	■	■	■				maturity stage - GSI	Montenrgrim Shelf	18
Lembo 2013				■	■	■	■	■					maturity stage	South Adriatic	18
Carbonara 2013				■	■	■	■	■					maturity stage	West Ionian	19
Karlou-Riga, 2000					■	■	■						GSI	Sarinokos Gulf	22
Demirel & Yüksek (2013)					■	■	■	■					maturity stage - GSI	Sea of Marmara	28

Considering the monthly percentage of maturity stages (AAVV, 2013) in the Central-Southern Tyrrhenian sea (GSA 10), South Adriatic sea (GSA 18) and West Ionian sea (GSA 19) the peak of spawning range between May and June (Carbonara, 2015).

The growth parameters reviewed in the Figure 2.3 showed a remarkable growth in the first three years of life followed by a slowdown maybe also due to the attainment of sexual maturity (Karlou-Riga, 2000; Zupa *et al.*, 2006; Belcari *et al.*, 2007; Nobile *et al.*, 2008). Moreover the differences of the growth pattern described by the several authors seem very high probably not only due to the geographical differences. For example following the growth model reviewed in the figure 2 at the end of the first year of life the Total length range between 10 cm in the Croatia coast (Šantic *et al.*, 2003) and 17.8 cm northern Tyrrhenian sea (Belcari *et al.*, 2007). The principal difficulties in the age interpretation are due by the identification of the first annulus, presences of the false ring and overlapping of winter ring in the oldest specimens.

Figure 2.3: The von Bertalanffy growth parameters obtained from literature.

2.3 Blue jack mackerel (*Trachurus picturatus*)

Trachurus picturatus (Bowdich, 1825) is a bentho-pelagic, schooling species, confined to neritic zones of island shelves, banks and seamounts. They can be found up to depth of 370 m.

FishBase (2015)

It is mainly distributed in the Eastern Atlantic: from the southern Bay of Biscay to southern Morocco including Azores, Madeira, the Canary Islands, Tristan de Cunha and Gough Islands. It is also found off Mauritania and in the Mediterranean Sea. Blue jack mackerel is known to migrate between the coast of Sahara and the offshore seamounts possibly reaching the Cape Verde Islands (Froese and Pauly, 2015).

As other small pelagic fish, they are commonly fished at night by artisanal purse-seiners. Larger specimens are caught in deeper waters, by bottom longline off Azores and Madeira archipelagos (Jesus, 1992; Garcia *et al.*, 2015), and even with pelagic trawls in Mauritanian waters (Jurado-Ruzafa *et al.*, 2011). Thus, each fishing gear affects to different fractions of the population.

FAO catches statistics of the species has clear data gaps. Usually, *T. picturatus* has been registered as *T. trachurus* and it is known that artisanal fisheries do not register data systematically. Therefore, this information is not useful. However, it is an important fishing resource for Azores, Madeira and the Canary Islands and regional registrations, although limited, are more reliable.

Regarding length-weight relationships, Table 2.2 shows the values obtained by several authors for *T. picturatus* from Atlantic areas. The mean allometric coefficient (*b*) from studies using Total Length (TL) is almost 3.2, which means that *T. picturatus* presents positive allometric growth. Information from Mediterranean Sea is not presented because results are not comparable due to the different methodology used.

Table 2.2: Summary of length-weight relationships of *T. picturatus* from the Atlantic area. M: males; F: females.

Area	Reference	Length	Group	N	Length-Weight relationships parameters		
					a	b	R²
S-SW Portugal	Borges *et al.* (2003)	TL	Total	45	0.0040	3.163	n.d.
W Portugal	Mendes *et al.*. (2004)	TL	Total	32	0.0068	3.041	n.d.
Azores	Isidro (1990)	FL	Defrost	419	0.0096	3.060	0.99
			Fresh	1934	0.0082	3.110	0.99
	Rosa *et al.* (2006)	FL	Total	211	0.0197	2.841	0.96
	Garcia *et al.* (2015)	FL	Total	2528	0.0090	3.087	0.99
			M	1333	0.0090	3.069	0.99
			F	1195	0.0080	3.113	0.99
Madeira	Jesus (1992)	TL	Total	3401	0.0044	3.201	0.99
	Vasconcelos *et al.* (2006)	TL	Total	621	0.0076	3.057	0.95
			M	95	0.0127	2.908	0.92
			F	126	0.0060	3.126	0.95
	Vasconcelos *et al.* (2008)	TL	Total	1005	0.0067	3.068	0.98
			M	448	0.0062	3.092	0.98
			F	494	0.0066	3.073	0.98
Canary Islands	Delgado de Molina *et al.* (1983)	TL	Total	557	0.0039	3.388	0.99
			M	84	0.0079	3.160	0.93
			F	35	0.0072	3.188	0.97
	Jurado-Ruzafa and Santamaría (2010)	TL	Total	2472	0.0023	3.417	0.97
			M	654	0.0048	3.184	0.98
			F	482	0.0036	3.287	0.98

Concerning the reproductive aspects, the sex ratio results are high variable depending on the temporal and spatial distribution. Spawning period occurs during winter and spring in Azores (Isidro, 1990; Garcia *et al.*, 2015), Madeira (Jesus, 1992; Faria and Vasconcelos, 2008), the Canary Islands (Jurado-Ruzafa and Santamaría, 2013) and the Sahara (Shaboneyev and Ryazantseva, 1977; Garcia, 1982), whereas it is delayed to summer in Moroccan (Gail, 1955; Garcia, 1982) and Mediterranean waters (Casaponsa, 1993 *in* Lloris and Moreno, 1995). First Maturity Length has been estimated only for the blue jack mackerel inhabiting these Atlantic archipelagos. The values for the total are almost similar for Madeiran and Canarian studies (21.5 and 23 cm TL, respectively) (Faria and Vasconcelos, 2008; Jurado-Ruzafa and Santamaría, 2013), and different compared with the Azorian result (28 cm fork length).

Growth parameters of blue jack mackerel obtained by different authors as well as the Index of growth performance (ɸ') are presented in Table 2.3. The maximum theoretical length ranged from 32.2 to 58.3 cm and the value of *k* between 0.07 and 0.316 (*T. picturatus* may grow to about 60 cm (Smith-Vaniz *et al.*, 1990)). This species grows rapidly during the first year of life and much more slowly after year 3-4. Maximum age is very variable among areas, being noticeable the ones obtained recently off Azores by Garcia *et al* (2015).

Table 2.3: Summary of growth parameters of _T. picturatus_. ML: from the paired values mean length per age (age-length keys); DR: from the total paired values of the direct readings; BC: from the paired values mean back calculated length per age. φ': Index of growth performance (Munro and Pauly, 1983).

Area	Reference	Method	Sex	N	Length	L_{inf} (cm)	k (years-1)	t_0 (years)	R^2	φ'	Age class (years) Min.	Age class (years) Max.	Length class (cm) Min.	Length class (cm) Max.
Azores	Isidro (1990)	ML	Total	516	FL	52.9	0.2	-0.23	n.a.	2.75	0	9	12	43
	Westhaus-Ekau and Ekau (1982)	DR	Total	n.a.	TL	51.1	0.144	-1.584	n.a.	2.57	n.a.	n.a.	n.a.	n.a.
	Garcia _et al._ (2015)	DR	Total	1420	FL	58.3	0.09	-2.67	n.a.	2.48	0	18	9	54
			♂	628	FL	64.4	0.07	-3.34	n.a.	2.46	n.a.	n.a.	10	54
			♀	489	FL	62.1	0.08	-3.11	n.a.	2.49	n.a.	n.a.	12	49
		BC	Total	796	FL	52.9	0.11	-2.45	n.a.	2.49	n.a.	n.a.	n.a.	n.a.
			♂	391	FL	60.2	0.08	-2.78	n.a.	2.46	n.a.	n.a.	n.a.	n.a.
			♀	320	FL	51.4	0.12	-2.18	n.a.	2.50	n.a.	n.a.	n.a.	n.a.
Madeira	Jesus (1992)	ML	Total	489	TL	44.3	0.316	n.d.	0.93	2.79	n.d.	n.d.	n.d.	n.d.
	Pereira (1993)	ML	Total	877	TL	43.8	0.23	-0.57	0.97	2.64	0	6	10	41
	Vasconcelos _et al._ (2006)	PV	Total	578	TL	42.3	0.161	-2.563	0.74	2.46	0	9	17	46
			♂	248	TL	39.6	0.194	-2.282	0.72	2.48	n.d.	n.d.	n.d.	n.d.
			♀	295	TL	49.8	0.114	-3.052	0.75	2.45	n.d.	n.d.	n.d.	n.d.
		BC	Total	229	TL	48.3	0.135	-2.898	0.99	2.50	n.a.	n.a.	n.a.	n.a.
			♂	89	TL	44.8	0.143	-3.207	0.99	2.46	n.a.	n.a.	n.a.	n.a.
			♀	131	TL	44.6	0.163	-2.43	0.99	2.51	n.a.	n.a.	n.a.	n.a.
	Vasconcelos _et al._ (2008)	ML	Total	889	TL	36.0	0.254	-1.445	0.99	2.52	0	5	13.3	36
			♂	391	TL	38.4	0.197	-1.994	0.99	2.46	n.a.	n.a.	n.a.	n.a.
			♀	454	TL	32.1	0.384	-0.764	0.99	2.60	n.a.	n.a.	n.a.	n.a.
Mediterranean Sea	Casaponsa (1993)	n.a.	Total	n.a.	TL	32.2	0.228	-1.469	n.d.	2.37	n.d.	n.d.	n.d.	n.d.
	Cefali _et al._ (2004)	n.a.	Total	875	FL	38.8	0.22	-1.79	n.d.	2.52	0	10	8	38
Canary Islands	Jurado-Ruzafa and Santamaria (2014)	DR	Total	913	TL	34.9	0.214	-2.545	0.87	2.42	0	6	10	31
			♂	695	TL	42.1	0.141	-3.038	0.87	2.40	0	5	10	31
			♀	625	TL	38.4	0.169	-2.835	0.87	2.40	0	6	10	29

Based on the growth parameters presented in Table 2.3, Natural Mortality means values were calculated through Hoenig and Jensen functions (Hoenig, 1983; Jensen, 1996), and ranged from 0.2 to 0.3 years-1.

There are many works addressing the analysis of parasites, genetics, etc. That could help to establish the abundance of each _Trachurus_ species and the proportion among them, as well as the stocks identification.

3 Review information on age determination, otolith exchanges and validation techniques on this species (ToR a)

3.1 Otolith exchanges and Workshops

Three exchanges and workshops have been executed to date (Table 3.1).

Table 3.1: Past _Trachurus_ species otolith workshops.

Year end	Exchange / workshop	Calcified pieces	Reference
1999	Workshop	Otolith section/ whole otolith	Report of the Horse Mackerel otolith Workshop 2006 ICES, 1999
2006	Workshop	Otolith section/ whole otolith	Report of the Horse Mackerel Exchange and Workshop 2006 Bolle et al., 2007
2012	Workshop	Otolith section/ whole otolith	No reference

3.2 Validation studies

Age determination of _Trachurus_ species is still a no simple matter. Data quality is a key issue in all ageing studies. In this way age estimates that are neither accurate nor precise would be of poor value for subsequent use. Validation studies that aim to verify the presumed periodicity of a given signal are essential basis for otolith aging studies, because they are the only way to test the technology and the accuracy of age estimation (Panfili _et al_., 2002). It is also essential to assess data precision to reveal the most appropriate schemes for reading and interpretation (Panfili _et al_., 2002). Theoretically a validation should be made of every population of any given species, since there may be important differences between them. There are few studies on validation of these three _Trachurus_ species being the majority of them related to horse mackerel _Trachurus trachurus_. Following the classification showed in Panfili _et al_. (2002), most of the validation studies belong to the classes of: semi-direct validation techniques (evolution of mark at the edge of the otolith, marginal increments); indirect validation techniques (comparison with length distributions, tracking of strong or weak year classes); and finally since late 1990's also the analysis of daily increments to interpret the otolith macrostructure.

3.2.1 Horse mackerel (*Trachurus trachurus*)

Using daily increment counts and marginal increment widths measures to estimate ages Waldron and Kerstan (2001, Figure 3.1) validated the ages of horse mackerel up to four years.

Figure 3.1: **Light micrograph illustrating a prominent ring on a small horse mackerel otolith aged using a light microscope (Bar=1 mm). Relationship between age (days) and otolith radius (mm) for South Atlantic (■) and North Atlantic (◊) horse mackerel (*In* Waldron and Kerstan, 2001).**

They analysed horse mackerels from Southeast Atlantic (*Trachurus capensis*) and from the Northeast Atlantic (*Trachurus trachurus*) and showed that false rings and annuli are often of a similar visual appearance. They stated that true annuli can only be identified if concurrent measurements of growth zone widths are available (the marginal increment method, Kerstan, 1985). However, most annuli in the whole otoliths could be distinguished from false rings by their sharper images and high contrast. They also confirmed that the estimated ages read from the whole otoliths are generally correct up to the age of four years.

In the review of Abaunza *et al.* (2003) appeared a section on the historical development of age interpretation criteria for the horse mackerel from the Northeast Atlantic. From this review we can extract the following milestones in relation with the validation process. Macer (1977) validated indirectly the ages for the first four years of life by using the length-frequency method and also demonstrated the occurrence of one translucent ring per annual growth zone. Similar results of indirect validation for the first years of life were obtained previously by other studies (Letaconnoux, 1951; Ramalho and Pinto, 1956; Barraca, 1963; Polonsky, 1969; Saharge, 1970). Various authors also recognized that otoliths from older fish became thicker with time and thus presented more difficulties for age determination (Macer, 1977; Alegria-Hernandez, 1984; Kerstan, 1985; Eltink and Kuiter, 1989). The appearance of the extremely strong 1982 year class in the Western stock (NE Atlantic) allowed defining with more cer-

tainty the age reading interpretation method of one translucent ring per annual growth zone. With this criterion and the application to the otoliths of the broken-burnt technique, the cohort was possible to track and then a kind of indirect validation also for adults or specimens older than four years old was obtained (Eltink and Kuiper, 1989).

There are almost no references for the case of age validation studies in *T. trachurus* from the Mediterranean Sea. Karlou-Riga and Sinis (1997) used marginal increments analysis, time of translucent formation, nature of otolith edge and the spawning period for age interpretation criteria with specimens from Greek waters. With the use of monthly samples during the first year of life they validated semi-directly the first annual ring. In general, these authors stated that the annuli were readable until the 5th. In the majority of their otoliths the first and second translucent rings appeared as single wide translucent zones. They validated the double formations of 3rd and 4th annuli, saying that the annuli for older fish might be double rings as well but due to the decrease of these annuli radii from the nucleus and to the increasing otolith thickness, these double formations were not always clear (Karlou-Riga and Sinis, 1997).

3.2.2   Mediterranean horse mackerel (*Trachurus mediterraneus*)

Biological data on Mediterranean horse mackerel are very limited and information on ageing accuracy is only found in Karlou-Riga (2000). This author applied the measurement of marginal increments to estimate the time of annulus formation and monthly sampling to obtain the length frequencies that allow following the progression of smaller fish modal length during the year. She stated that: a) false annuli, usually identifiable before the *antirostrum*, is formed, and that this sometimes led to confusion of the first annulus interpretation; b) In general, the opaque area was very narrow and do not show a clear contrast to the translucent one; c) Translucent rings for the same age could be single or multi-ring zones and d) Some otoliths had an equally spaced multi-ring structure. Karlou-Riga (2000) also interpreted that successive translucent rings were annuli on the basis of their formation at progressively greater distances from the nucleus. The time of annulus completions was found to be around the end of autumn and coincided with the end of the spawning period in the Saronikos Gulf (Karlou-Riga, 2000).

3.2.3   Blue jack mackerel (*Trachurus picturatus*)

There is also very scarce information on the accuracy of age estimation in this species. At this workshop there is only available the work of Pereira de Gouveia (1993) with blue jack mackerels captured from Madeira Island. She applied the method of Bhattacharya to length frequency distributions as a way to validate ageing. She obtained five modal components corresponding to ages "0" to "IV". She stated the following criteria for age interpretation: one translucent ring per year, true annuli are wider than possible false annuli. There is a decrease in the distance between the old and the new annuli as fish is getting older. In this way she obtained specimens up to six years of age.

4   Review of ageing techniques

During the WKARHOM2 meeting, the data compiled by the WKNARC 1 meeting (ICES, 2011) was extracted and updated. All institutes used otolith as ageing structures for *Trachurus* species but there are many different preparation methods (Table 4.1, Annex 4).

Table 4.1: Preparation methods of otoliths used for *Trachurus* species ageing by institutes and areas.

Ecoregion	IES Divisions	*T. mediterraneus* section	*T. mediterraneus* whole	*T. picturatus* whole	*T. trachurus* section	*T. trachurus* whole	*T. trachurus* stained section	*T. trachurus* whole or break and polish	*T. trachurus* burning break	*T. trachurus* polishing
Barents Sea	27.1a,1b/2a2,2b2								1	
North Sea	27.3a,4,7d				1	1	1	2	1	
Norwegian Sea	27.2a1,2a2								1	
Celtic sea	27.6a,6b,6b1,6b2,7a,7b1, 7b2,7c1,7c2,7e,7f,7g,7h, 7j2,7k2				1	1	1	2		
Western Mediterranean Sea	37.1.1,1.2,1.3		4		1	3				1
Adriatic-Ionian Seas	37.2.1, 2.2		1		1					1
Oceanic northeast Atlantic	37.7k1,8d1,8d2,8e1,8e2, 9b1,9b2, 10a1,10a2,12a1,12a3,12 a4,12b,12c			1						
South European Atlantic Shelf	37.8abc,9a	1			2	1			1	
Total général		1	5	1	6	6	2	4	4	2

During the WARHOM2 meeting, the conclusion of discussion identify that there are only difference of preparation methods for *Trachurus* ageing in the Atlantic Ocean. For the others *Trachurus* species (*T. mediterranneus* and *T. picturatus*), the whole otolith is used to ageing.

5 Estimate (relative) accuracy and precision of horse mackerel, Mediterranean horse mackerel and blue jack mackerel age determination in the main European fishing areas (ToR b)

The main results of the exchanges 2012 and 2014 (Mahé *et al.*, 2015) are presented in this part of the report.

The spreadsheet (Eltink, 2000) was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink *et al.* (2000). Modal ages were calculated for each otolith red, with percentage agreement, mean age and precision coefficient of variation as a definition (for each otolith):

- Percentage agreement = 100x (no. of readers agreeing with modal age/total no. of readers).
- Precision c. v. = 100x (standard deviation of age readings/mean of age readings).

5.1 Exchange 2012

16 age readers participated in the otolith exchange, seven of the institutions read sectioned otoliths, three read whole otoliths, two read broken burnt whole otoliths and 3 read sectioned otoliths and whole otoliths.

There were ten sets of images of *Trachurus trachurus*, *T. mediterreaneus* and *T. picturatus* (Table 5.1). Exchanged otoliths were from Ireland, North Spain, South Spain, Azores, Mauritania and Adriatic Sea.

Table 5.1: Overview of ten sets of images with areas of sampling, species and otolith preparation in the *Trachurus* otolith exchange 2012.

SET NUMBER	AREAS	SPECIES	PREPARATIONS
1	North of Spain	*T. trachurus*	Slice
2	Ireland	*T. trachurus*	Slice
3	Portugal	*T. trachurus*	Slice
4	South of Spain	*T. trachurus*	Slice
5	Western Ireland	*T. trachurus*	Slice
6	Western Ireland	*T. trachurus*	Slice
7	Italy	*T. mediterraneus*	Whole
8	Spain	*T. mediterraneus*	Whole
9	Azores	*T. picturatus*	Whole
10	Mauritania	*T. picturatus*	Whole

5.1.1 Precision

Mean precision[1] of age estimate for individual fish were calculated by Coefficient of Variation (CV) and percent agreement to modal age. There were little variations in

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[1] Precision is defined as the variability in the age readings. The precision's errors in age readings fare better described by the coefficient of variation (CV) by age group. This measure of precision is independent of the closeness to the true age (ICES, 2007)

precision of age estimate among different sets, with CV ranging from 21 to 42.7% and percent agreement range from 36.4 to 67 % (Table 5.2). Consequently, these results show that the ages of these three species are also difficult to interpret. Moreover, there are no big differences among areas.

Table 5.2: Overview of 10 sets of images with number of readers, number of images, percentage of agreement and CV in the *Trachurus* otolith exchange 2012.

SET NUMBER	READERS NUMBER	IMAGES NUMBER	% AGREEMENT	CV
1	11	63	53.2	42.3
2	11	22	36.4	26.9
3	7	25	44.7	54.7
4	13	40	43.9	43.8
5	15	48	46.4	21
6	15	44	43.7	25.5
7	7	70	56.6	28.7
8	8	23	57.5	30.5
9	9	30	67	32.3
10	8	17	49.5	31.8

The sets 5 and 6 of *T. trachurus* in the western Ireland were interpreted during the two previous exchanges (Table 5.3). During three exchanges, these sets were interpreted with percentage of agreement close to 50%.

Table 5.3: Overall agreement with modal age in 3 exchanges by year and number of set with the number of readers who participated in the exchange.

YEAR OF EXCHANGE	NUMBER OF SET	READERS NUMBER	IMAGES NUMBER	% AGREEMENT
1999	5 (set G)	15	170	42
2006	5 (set G)	9	48	53
2012	5 (set G)	15	48	46.4
1999	6 (set K)	15	153	49
2006	6 (set K)	9	48	56
2012	6 (set K)	15	44	43.7

5.1.2 Accuracy

Sets 5 and 6 consisted of otoliths from the extremely strong 1982 year class and hence the age is considered to be known (with a certainty of approximately 95%). These sets have been used in the previous horse mackerel workshop (ICES, 1999) and were therefore renumbered for this exchange.

For the sectioned otoliths of sets 5 and 6, the results from all readers showed an improvement to subset experienced readers (Table 5.4).

Table 5.4: Overall agreement with 'true' age in exchange 2006 and 2012 for the images sets 5 and 6. In 2012 exchange, the results are presented with all readers who participated in both the workshop and with only 8 expert readers.

Year of exchange	Number of set	Readers number	Images number	% Agreement
2006	5 (set G)	9	48	43
2012	5 (set G)	15 (all readers)	48	41.5
2012	5 (set G)	8 (expert readers)	48	54.3
2006	6 (set K)	9	48	36
2012	6 (set K)	15 (all readers)	44	38.2
2012	6 (set K)	8 (expert readers)	44	51.2

5.2 Exchange 2014

5.2.1 Sampling collection

A total of 550 fish was sampled from 2003 to 2014 (2003, 2004, 2005, 2006, 2007, 2009, 2011 and 2014) from both Atlantic and Mediterranean area (Table 5.5; Figure 5.1):

- 95 *Trachurus mediterraneus* sampled in the three geographical areas by COISPA (Italy) and IEO (Spain)
- 134 *Trachurus picturatus* sampled in the three geographical areas by IEO (Spain), COISPA (Italy) and DOP (Portugal)
- 321 *Trachurus trachurus* sampled in the three geographical areas by Ifremer (France), DISTAV (Italy), TI-SF (Germany) and IEO (Spain)

Table 5.5: Samples distribution by *Trachurus* species (number corresponding to map)

Species	Atlantic Ocean						Mediterranean Sea			Total
	Eastern Channel VIId (1)	Celtic Sea VIIh (2)	Bay of Biscay VIIIc (3)	Azores XII (4)	Portuguese waters IXa (5)	Tenerife (6)	Alboran Sea (7)	South Adriatic Sea (8)	Ligurian Sea (9)	
T. mediterraneus			35		10			50		95
T. picturatus				71		50		13		134
T. trachurus	50	154					20		97	321

Figure 5.1: Map of *Trachurus* species sampling areas (*Source*: Google Map 2015).

The samplings came from both the harbour and the survey. The otolith sections and whole otoliths were used during the 2014/2015 exchange but only *Trachurus trachurus* was sampled with both techniques (Table 5.6).

Table 5.6: Samples distribution by *Trachurus* species and by preparation method of otolith.

SPECIES	OTOLITH SECTION	WHOLE OTOLITH
Trachurus mediterraneus		95
Trachurus picturatus		134
Trachurus trachurus	201	120
Total	154	349

5.2.2 Reading procedure

One image of each otolith was uploaded to WebGR (http://webgr.azti.es/ce/search/myce). All participants received all information to participate to this exercise in the WebGR tool. The WebGR tool was used to this exchange. The use of WebGR tool for the exchange has some advantages: (i) it can facilitate and accelerate the whole exchange process, (ii) annotated images are obtained for every otolith which allows to compare age readings directly and to identify possible sources of bias (iii) it is very easy for the Chair to compile the results. However, the use of WebGR tool for the exchange present some limits: (i) the WebGR tool is not very intuitive tool (ii) the WebGR could be jammed (iii) it is not always possible to upload a large batch of images (problem with the format of the csv file with Windows 7).

The age was assigned taking into account the number of the transparent rings. Moreover the date of capture, the sex and total length were visible by the readers.

All data were extracted of the WebGR.

5.2.3 Precision

The precision[2] analyse with Coefficient of Variation (CV) and percent of agreement to modal age for otoliths sets according to the *Trachurus* species was presented in the Table 5.7. Among three *Trachurus* species, all data showed the very low precision with the percent agreement between 47 and 56% and the CV from 29 to 69 (Table 5.7).

Table 5.7: Reading's precision by *Trachurus* species.

TRACHURUS SPECIES	OTOLITHS NUMBER	READERS NUMBER	AGE RANGE	PERCENTAGE OF AGREEMENT	CV
T. mediterraneus	95	15	0/12	47.1%	43.8%
T. picturatus	133	13	0/15	48.9%	69.0%
T. trachurus	309	17	0/15	55.8%	28.7%

5.2.4 *Trachurus mediterraneus*

The results of otoliths readings of *Trachurus mediterraneus* showed a better precision in the Mediterranean area than those obtained in the sampling areas from the Atlantic Ocean (Table 5.8).

Table 5.8: Reading's precision of *Trachurus mediterraneus* by sampling areas.

SAMPLING AREAS	OTOLITHS NUMBER	READERS NUMBER	AGE RANGE	PERCENTAGE OF AGREEMENT	CV
VIIIc	35	15	0/10	39.3%	40.2%
IXa	10	15	0/6	41.2%	41.7%
South Adriatic Sea	50	15	0/12	53.6%	46.7%

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[2] Precision is defined as the variability in the age readings. The precision's errors in age readings are better described by the coefficient of variation (CV) by age group. This measure of precision is independent of the closeness to the true age (ICES, 2007).

5.2.4.1 Trachurus picturatus

The results of otoliths readings of *Trachurus picturatus* showed a lower precision in the Azores area than those obtained in the other sampling areas due to especially the old specimen in this area (Table 5.9).

Table 5.9: Reading's precision by *Trachurus picturatus* **by sampling areas.**

SAMPLING AREAS	OTOLITHS NUMBER	READERS NUMBER	AGE RANGE	PERCENTAGE OF AGREEMENT	CV
Azores	70	13	0/15	35.3%	36.0%
Tenerife	50	12	0/5	60.1%	89.3%
South Adriatic Sea	13	12	0/1	79.3%	168.8%

5.2.4.2 Trachurus trachurus

The results of otoliths readings of *Trachurus trachurus* showed a better precision in the Atlantic Ocean (VIId and VIIh) than those obtained in the sampling areas from the Mediterranean Sea (Alboran and Ligurian Seas) (Table 5.10). Moreover, in the Ligurian Sea, the analysis between otoliths section and whole otoliths showed the same level of precision (Table 5.10).

Table 5.10: Reading's precision by *Trachurus trachurus* **by sampling areas.**

SAMPLING AREAS	OTOLITHS NUMBER	OTOLITH PREPARATION	READERS NUMBER	AGE RANGE	PERCENTAGE OF AGREEMENT	CV
VIId	50	Section	16	2/15	55.7%	16.8%
VIIh	154	Section	16	1/14	63.8%	25.9%
Alboran Sea	20	Whole	17	1/3	50.1%	69.7%
Ligurian Sea	45	Section	13	1/14	44.6%	32.6%
Ligurian Sea	50	Whole	14	1/10	44.0%	28.9%

5.2.5 Accuracy

The minimal requirement for age reading's consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated can be tested non-parametrically with a one-sample Wilcoxon signed rank test.

5.2.5.1 Trachurus mediterraneus

It should be noted that there are certainly of bias between some readers and modal age for *T. mediterraneus* otoliths. There were no observed bias between five readers (5/15 readers, 33%) and the modal age (Table 5.11).

Table 5.11: Inter-reader bias test and reader against modal age bias test of *Trachurus mediterraneus* otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

Inter-reader bias test and reader against MODAL age bias test

	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	France Reader 9	Italy Reader 10	Germany Reader 12	Ireland Reader 6	Portugal Reader 2	Portugal Reader 8	Spain Reader 4	Spain Reader 5	Italy Reader 7	Spain Reader 11	Italy Reader 13
Reader 15															
Reader 1	**														
Reader 14	**	**													
Reader 3	*	—	**												
Reader 9	**	*	**	**											
Reader 10	**	**	**	**	—										
Reader 12	*	**	**	**	**	**									
Reader 6	—	**	**	**	**	**	—								
Reader 2	**	**	**	**	—	—	**	**							
Reader 8	—	**	**	*	**	**	**	**	**						
Reader 4	*	**	**	**	**	**	—	—	**	**					
Reader 5	—	**	**	*	**	**	—	**	**	—	*				
Reader 7	**	—	**	*	—	**	**	**	—	**	**	**			
Reader 11	—	**	**	**	**	**	**	*	**	*	*	*	**		
Reader 13	—	**	**	—	**	**	**	**	**	—	**	*	**	**	
MODAL age	—	**	**	—	**	**	**	**	**	—	**	—	**	**	—

5.2.5.2 *Trachurus picturatus*

It should be noted that there are certainly of bias between some readers and modal age for *T. picturatus* otoliths. There were no observed bias between two readers (2/13 readers, 15%) and the modal age (Table 5.12).

Table 5.12: Inter-reader bias test and reader against modal age bias test of *Trachurus mediterraneus* otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

Inter-reader bias test and reader against MODAL age bias test

	Germany Reader 12	Spain Reader 4	France Reader 9	Portugal Reader 8	Portugal Reader 2	Italy Reader 10	Italy Reader 3	Ireland Reader 6	Ireland Reader 14	Spain Reader 1	Spain Reader 5	Italy Reader 7	Spain Reader 11
Reader 12													
Reader 4	**												
Reader 9	—	**											
Reader 8	**	**	**										
Reader 2	**	**	**	**									
Reader 10	**	**	**	**	*								
Reader 3	*	**	—	**	**	**							
Reader 6	**	—	**	**	—	**	**						
Reader 14	**	**	**	**		**	**	*					
Reader 1	—	**	—	**	**	**	—	**	**				
Reader 5	**	**	**	**	**	**	**	**	**	**			
Reader 7	**	**	**	**	**	—	**	**	**	**	**		
Reader 11	**	**	**	**	**	**	**	*	**	**	**	**	
MODAL age	*	**	**	**	**	**	—	**	**	**	—	**	**

5.2.5.3 *Trachurus trachurus*

It should be noted that there are certainly of bias between some readers and modal age for *T. mediterraneus* otoliths. There were no observed bias between five readers (5/17 readers, 29%) and the modal age (Table 5.13).

Table 5.13: Inter-reader bias test and reader against modal age bias test of *Trachurus trachurus* otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

Inter-reader bias test and reader against MODAL age bias test

	France Reader 9	France Reader 17	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	Germany Reader 12	Ireland Reader 6	Netherland Reader 16	Spain Reader 11	Spain Reader 4	Portugal Reader 2	Portugal Reader 8	Spain Reader 5	Italy Reader 7	Norway Reader 19	Italy Reader 13
Reader 9																	
Reader 17	**																
Reader 15	**	**															
Reader 1	**	**	**														
Reader 14	**	**	**	**													
Reader 3	**	*	**	—	**												
Reader 12	**	**	**	**	**	**											
Reader 6	**	**	**	—	**	—	**										
Reader 16	**	**	**	—	**	—	**	—									
Reader 11	**	**	**	**	**	**	**	**	**								
Reader 4	**	**	**	**	**	**	**	**	**	*							
Reader 2	**	**	**	—	**	**	**	**	**	**	**						
Reader 8	**	**	**	**	**	—	**	**	**	**	**	**					
Reader 5	**	*	**	**	**	—	**	**	*	**	**	—	**				
Reader 7	**	—	**	**	**	**	**	**	**	**	**	**	**				
Reader 19	**	**	**	**	**	**	**	**	**	**	**	—	**	**			
Reader 13	**	**	—	**	**	**	**	**	**	**	**	**	**	**	**		
MODAL age	**	**	**	—	**	—	**	—	—	**	**	**	—	**	**	**	**

5.2.6 Conclusions

A total of 550 fish was sampled from the Atlantic Ocean (Eastern Channel, Celtic Sea, Bay of Biscay, Azores, Portuguese waters and Tenerife) and the Mediterranean Sea (Alboran Sea, South Adriatic Sea and Ligurian Sea). 19 readers from 8 countries (France, Germany, Spain, Ireland, Italy, Portugal, Netherlands and Norway) participated to this exchange. Among three *Trachurus* species, all data showed the very low precision with the percent agreement between 47 and 56% and the CV from 29 to 69%. The precision analysis showed the same level of precision between otolith sections and whole otoliths from the Ligurian Sea.

5.3 New exercise

After having discussed all together on the images with annotations from the last exchange, a new exercise of reading was realized from a total of 120 images:

- 30 images of whole otoliths of *T. trachurus*
- 30 images of slices of *T. trachurus*
- 30 images of whole otoliths of *T. mediterraneus*
- 30 images of whole otoliths of *T. picturatus*

The WebGR tool was not used because it was not possible to select the images in the new workshop. Each reader read only the sets of these species.

The progress of reading showed the percent agreement close to 65% for *Trachurus trachurus* and *Trachurus picturatus*. However, the percent agreement for *Trachurus mediteraneus* remained to 44.4% with CV to 40 (Table 5.14). In fact, the next exchange must be target *Trachurus mediterraneus* as a priority.

Table 5.14: Reading's precision by *Trachurus* species and preparation methods.

	T. TRACHURUS	T. TRACHURUS	T. MEDITERRANEUS	T. PICTURATUS
	Slices	Whole otolith	Whole otolith	Whole otolith
% Agreement	65.7	65.8	44.4	63.3
CV	25	27.5	40	43.4
Readers number	12	12	10	9

6 Identify causes of age determination error and provide species-specific guidelines for the improvement of precision and reduction of bias between readers and laboratories (ToR c)

6.1 Otolith section *vs* whole otolith

6.1.1 During the WKARHOM1 meeting

To compare 3 techniques (whole otolith, whole otolith with thymol and slice; Figure 7.1), a set of 30 otoliths prepared by Ipimar institute was interpreted by all readers during the WKARHOM1 meeting. Consequently, 90 images (30 per each technique) were interpreted by all readers (Figure 6.1).
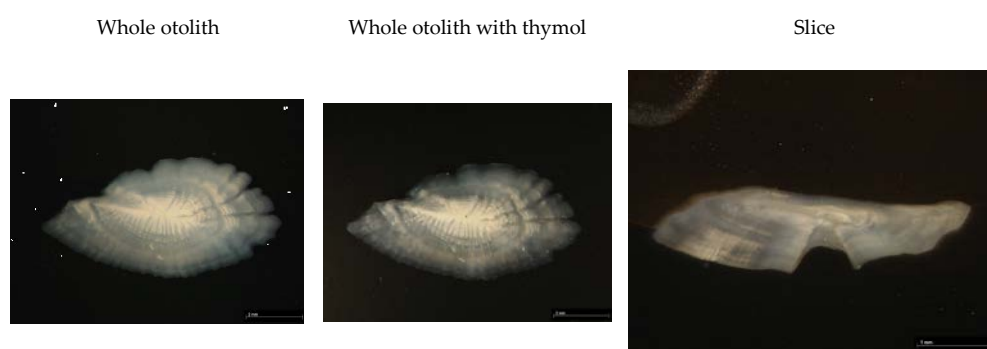
| Whole otolith | Whole otolith with thymol | Slice |

Figure 6.1: Otolith of *Trachurus trachurus*: whole, whole with thymol and slice.

3 techniques showed the same level of precision which was represented by Coefficient of Variation (CV) and percent agreement to modal age among all readers (Table 6.1).

Table 6.1: % agreement and CV of Trachurus trachurus readings by ageing technique during the exchange 2012.

TECHNIQUES	% AGREEMENT	CV
Whole otolith	53.8	37.3
Whole otolith with thymol	49.5	38.8
Slice	49	38.5

By comparing the techniques, percent agreement to modal age and Coefficient of Variation (CV) were respectively of 82.2% and 11.1%. However, there is necessary to remain careful because the sample was made up only of individuals between one and seven years of modal age (Figure 6.2).

Among the 30 images, only 6 had different modal ages between whole otolith with and without thymol. This number increased to 11 (37%) between whole otolith and slice. Age bias plots by preparation technique showed that there were differences in interpretation primarily on the old individuals. Estimated age from slice was higher than this from whole otolith (Figure 6.2).
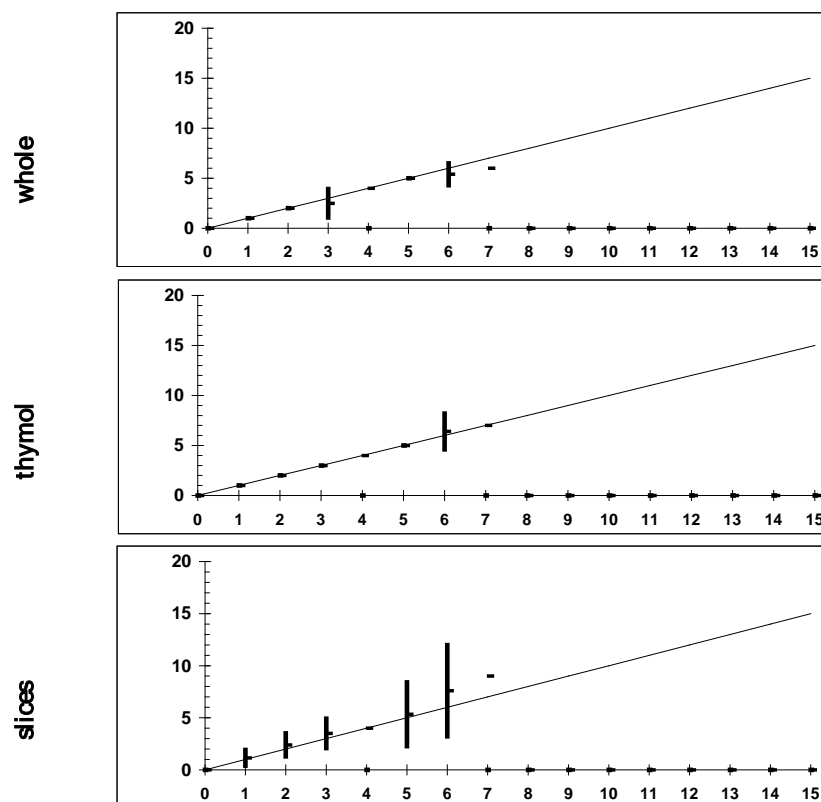
Figure 6.2: Age bias plots with the mean age recorded +/- 2stdev of each age by technique (The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line)).

6.1.2 During the exchange 2014

During the last exchange, the two sets of *Trachurus trachurus* (N=48) were realized from the same fish to evaluate the bias between ageing techniques. The results showed the same level of precision as the other exercise (Table 6.2).

Table 6.2: % agreement and CV of *Trachurus trachurus* readings by ageing technique during the exchange 2014.

TECHNIQUES	% AGREEMENT	CV
Whole otolith	44.6	28.9
Slice	44.9	32.6

Age bias plots by preparation technique showed that there were differences in interpretation on all age groups (Figure 6.3) and there is no pattern as estimated age from slice was higher than this from whole otolith observed in the first exercise.
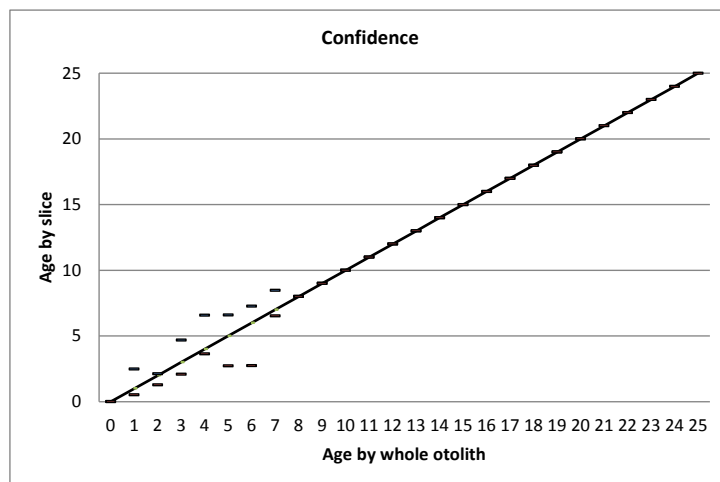
Figure 6.3: Age bias plots of each age by preparation technique of *Trachurus trachurus* in the Ligurian Sea (N=48; if the estimated mean age is on the 1:1 equilibrium line (solid line)).

For each reader, the results shown the significant difference between the ages estimated by two ageing techniques (Table 6.3).

Table 6.3: Bias, coefficient of variation (CV), percent agreement (PA) and absolute percent error (APE) % of *Trachurus trachurus* readings by ageing technique for each reader during the exchange 2014.

	France	Spain	Spain	Italy	Germany	Ireland	Netherlands	Spain	Spain	Portugal	Spain	Italy	Norway	All readers
	Reader 9	Reader 15	Reader 1	Reader 3	Reader 12	Reader 6	Reader 16	Reader 11	Reader 4	Reader 8	Reader 5	Reader 7	Reader 19	
No. Aged	45	37	20	31	43	47	47	47	46	41	43	41	44	47
No. Agreed	14	7	6	11	13	20	11	13	12	23	15	21	17	13
No. Diff.	31	30	14	20	30	27	36	34	34	18	28	20	27	34
Bias	-0,356	-0,838	-2,7	-0,161	0,07	0,234	-0,532	0,681	0,565	-0,61	0,186	-0,073	0	0,319
CV	0,195	0,123	0,63	0,246	0,19	0,156	0,216	0,125	0,144	0,094	0,162	0,157	0,182	0,241
APE	33,78	38,68	28,38	36,87	44,33	23,88	34,49	31,05	30,1	11,57	26,71	19,63	26,25	36,17
% Agreed	31,11	18,92	30	35,48	30,23	42,55	23,4	27,66	26,09	56,1	34,88	51,22	38,64	27,66

6.1.3 Conclusions

The results of the comparison between different ageing techniques on the same set of fish, showed a bias intra-reader and so it was recommended to use only one ageing technique by each reader. Moreover, the precision of reading is the same between slices and whole otoliths and so there is not a best ageing technique for *T. trachurus.*

6.2 Causes of age determination error identified during the WKARHOM2 meeting

During the workshop we had a plenary discussion regarding the first exchange (Mahé *et al*., 2014) analysing some images from the different set by species. During this section the images with a lower agreement was discussed for the identification the most important bias that they affected the accuracy (% agreement and CV).

6.2.1 *Trachurus trachurus* images

EB_14_B7_C6_OR001 (agreement: 27%; CV 25%; modal age: 7; age from readers: 4–10 years; Length: 35 cm; month of capture: April)

The posterior area is characterized by many multiple rings.

EB_14_B7_C3_OR001 (agreement: 20%; CV 25%; modal age: 7; age from readers: 4–10 years; Length: 35 cm; month of capture: April)

The pattern of deposition of the first rings was quite clear, but the considerably over-lapping of the rings (transparent and opaque) start from the 4 -5th winter ring.
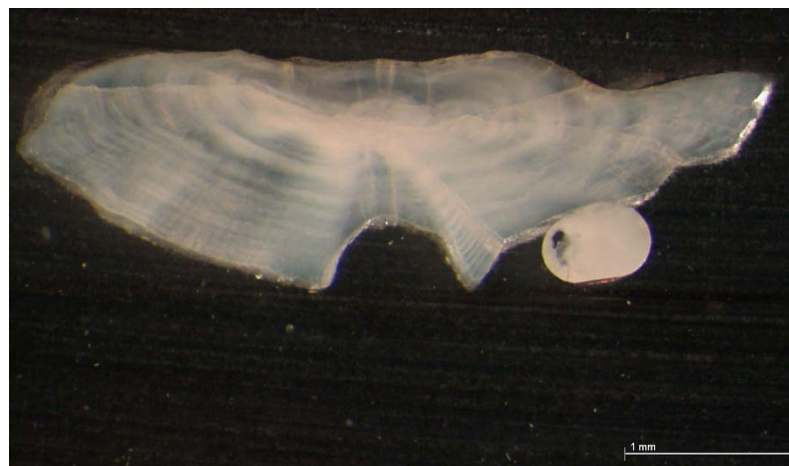
EB_14_B6_C1_OC0002 (agreement: 19%; CV 31%; modal age: 7; age from readers: 6–17 years; Length: 31 cm; month of capture: April)

This sample is thin section with a transmitted light; the preferred reading area is around the *sulcus acusticu*s, moreover mostly readers usually read the thin section by reflected light and no by transmitted light.

Stocker 81 (agreement: 29%; CV 36%; modal age: 2; age from readers: 2–6 years; Length: 24.5 cm; month of capture: November)

There is a disagreement on the position of the first ring.

EB_14_B6_C1_OCR0003 (agreement: 40%; CV 14%; modal age: 14; age from readers: 6–15 years; Length: 35.5 cm; month of capture: April)
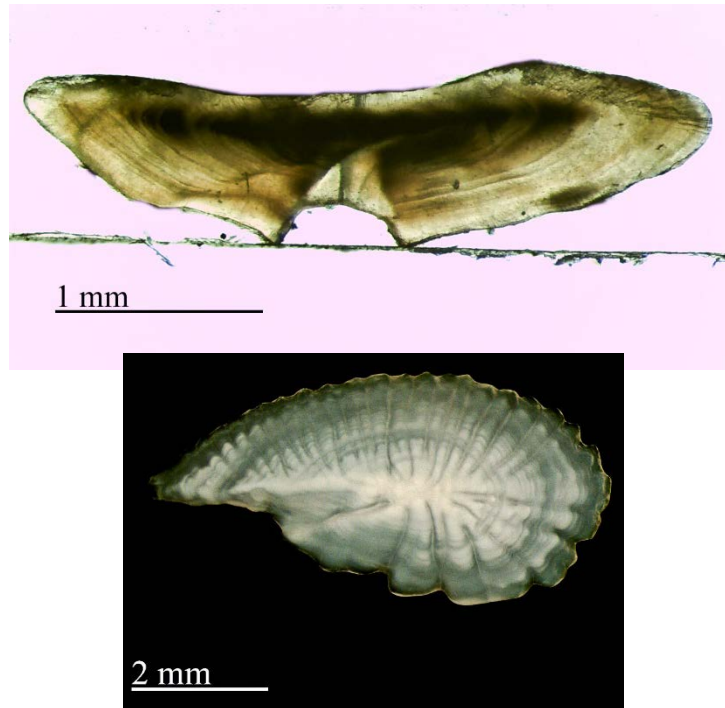
The pattern of deposition of the first rings was quite clear, but the considerably over-lapping of the rings (transparent and opaque) start from the 5th winter ring.

EB_14_B6_C2_OR0001 whole-right (agreement: 41%; CV 17%; modal age: 6; age from readers: 4–7 years; Length: 28 cm; month of capture: April)

EB_14_B6_C2_OR0001 slide-left (agreement: 81%; CV 7%; modal age: 6; age from readers: 5–7 years; Length: 28 cm; month of capture: April)
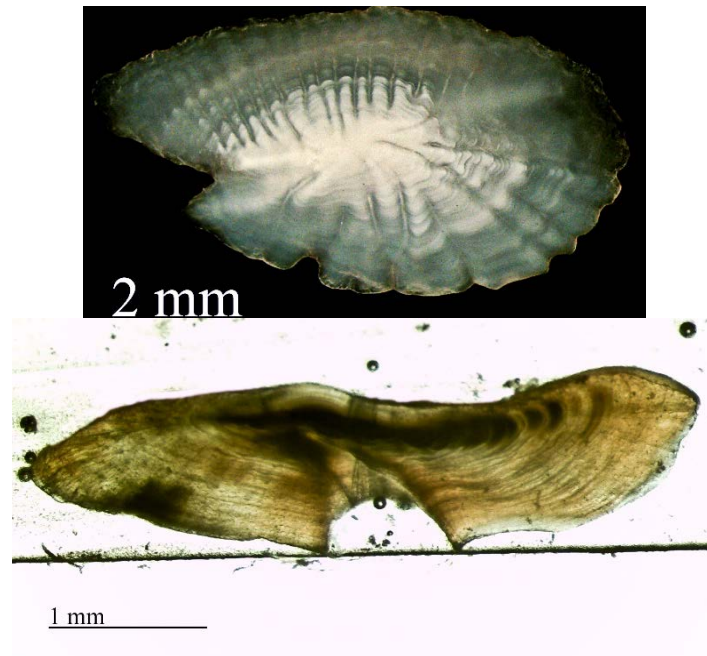
These two images come from the same specimen: one whole and other slide. In the slide the growth pattern was clear the differences are for the disagreement on the nature of the edge (opaque or translucent). Moreover, in the whole otolith the source of bias is represented by the presence of the reproductive ring after the second winter ring. Regarding the methodology half of the readers routinely use the slide and they analyse the slide with reflected light with a black background.

TT_S_0211 slide-left (agreement: 46%; CV 37%; modal age: 2; age from readers: 1–3 years; Length: 20 cm; month of capture: December)

TT_W_0211 whole-right (agreement: 64%; CV 19%; modal age: 3; age from readers: 2–3 years; Length: 20 cm; month of capture: December)

These two images come from the same specimen: one whole and other slide. The slide was with transmitted light and nobody use this technique routinely for the age analysis. In the whole there was a disagreement for the first winter ring. The higher agreement is in the whole otolith.

TT_S_0111 slide-left (agreement: 45%; CV 38%; modal age: 9; age from readers: 3–12 years; Length: 31 cm; month of capture: November)

TT_W_0211 whole-right (agreement: 64%; CV 19%; modal age: 3; age from readers: 2–3 years; Length: 31 cm; month of capture: November).

These two images come from the same specimen: one whole and other slide. The slide was with transmitted light and nobody use this technique routinely for the age analysis, moreover the slide seems too thin and the transmitted light tends to reduce the differences between the rings and their identification. In the whole there was a disagreement for the first winter ring. The higher agreement is in the whole otolith even if the presence of the decalcification area in the posterior part makes difficult the ring reading.
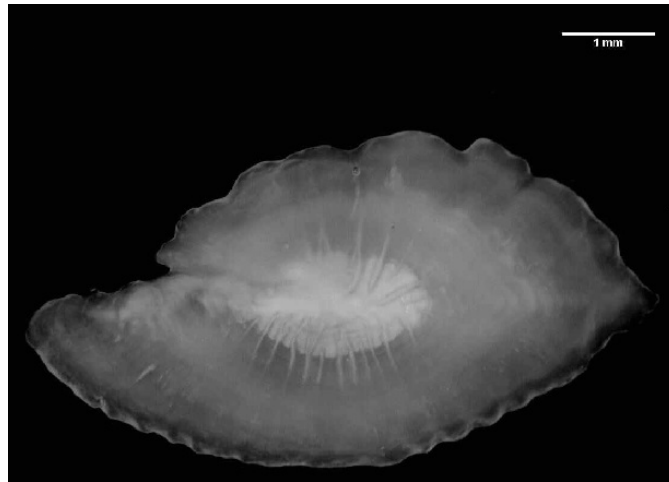
Conclusion

For the *T. trachurus* the main source of bias in the ageing analysis are:

- The identification of the first winter ring for the presence of some false mark before the deposition of the first annulus;
- The overlapping of the transparent ring after the 4-5th winter ring. Indeed distance between the ring after the first 4-5 rings decrease as much that it is very difficult to follow the rings around the perimeter of the otolith and then count them.

Regarding the preparation the slide in the case of the pictures with reflected light presented higher agreement than the whole one, while in the case of slide with transmitted light the whole otolith had the higher agreement. Then the participants of the exchange suggest using the reflected light for the analysis of the slide. Regarding the thickness of the slide about 0.5 mm seems give the best results.

6.2.2   *Trachurus mediterraneus* images

COISPA_25 (agreement: 73%; CV 24%; modal age: 3; age from readers: 1–4 years; Length: 21.5 cm; month of capture: March)

The main source of the bias came from the disagreement on the first winter ring.

MHM_P_27032011_L1_27_12 (agreement: 64%; CV 34%; modal age: 2; age from readers: 2–5 years; Length: 21.3 cm; month of capture: March)

The main source of the bias in this case comes from to the identification of the first winter ring.
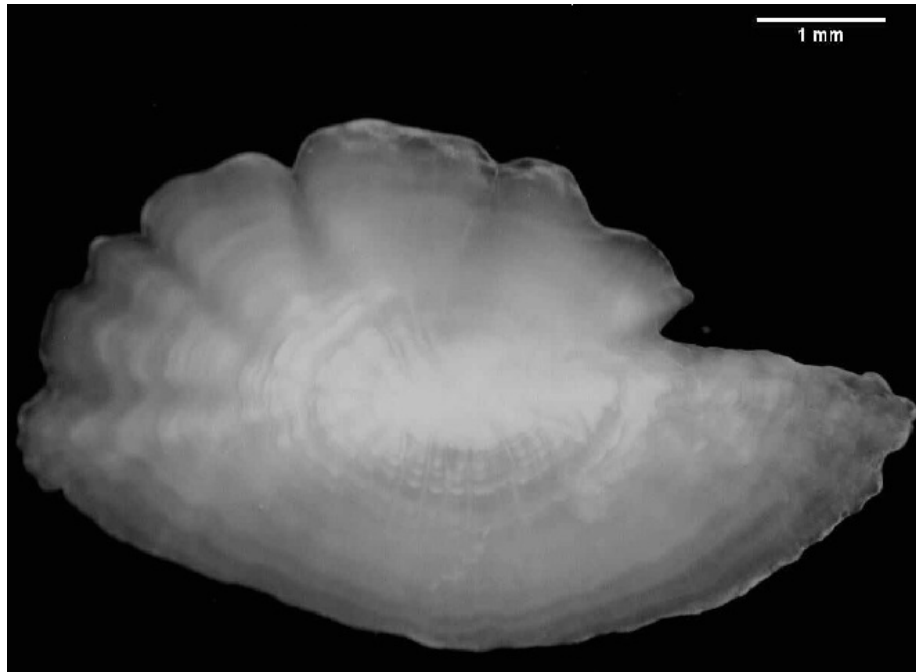
MHM_P_16042011_L38_15_18 (agreement: 58%; CV 23%; modal age: 3; age from readers: 2–5 years; Length: 24.7 cm; month of capture: April)

Disagreement on the second and third winter rings.
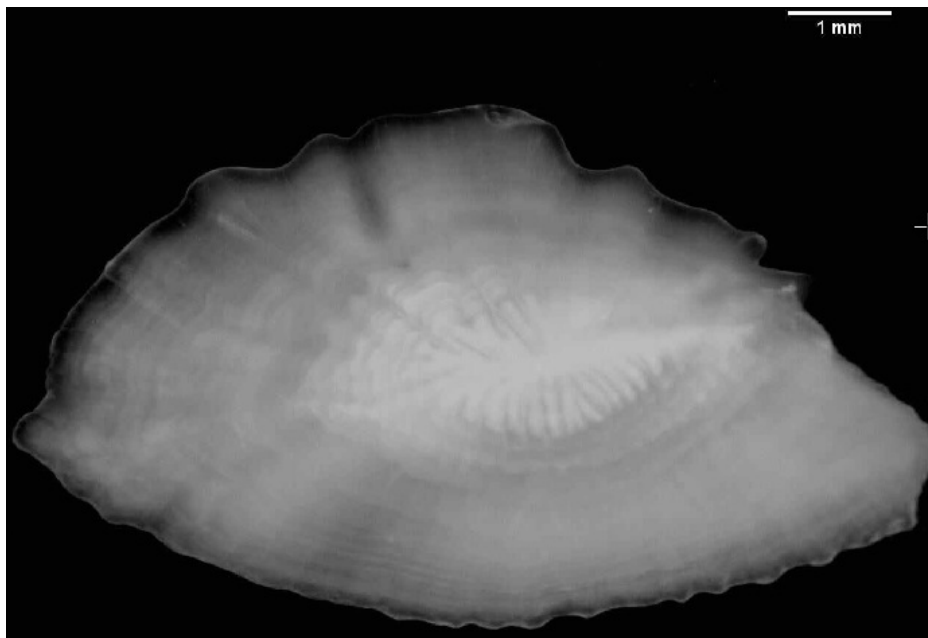

COISPA_50 (agreement: 47%; CV 11%; modal age: 10; age from readers: 8–12 years; Length: 35.5 cm; month of capture: March)

The main source of the bias in this case comes from to the identification of the first winter ring.

COISPA_2 (agreement: 27%; CV 36%; modal age: 3; age from readers: 3–9 years; Length: 23 cm; month of capture: July)

The main source of the bias in this case comes from to the identification of the first winter ring. The distance from the core to the first evident ring seems more or less the same of the distance from the first ring to second one.

COISPA_4 (agreement: 29%; CV 42%; modal age: 3; age from readers: 2–14 years; Length: 41.5 cm; month of capture: July)

In this case there are several source of bias. The disagreement in the identification of the first winter ring, the presence of false ring (between the first and second ring), and the overlapping of the ring on the edge are the principal source of bias.
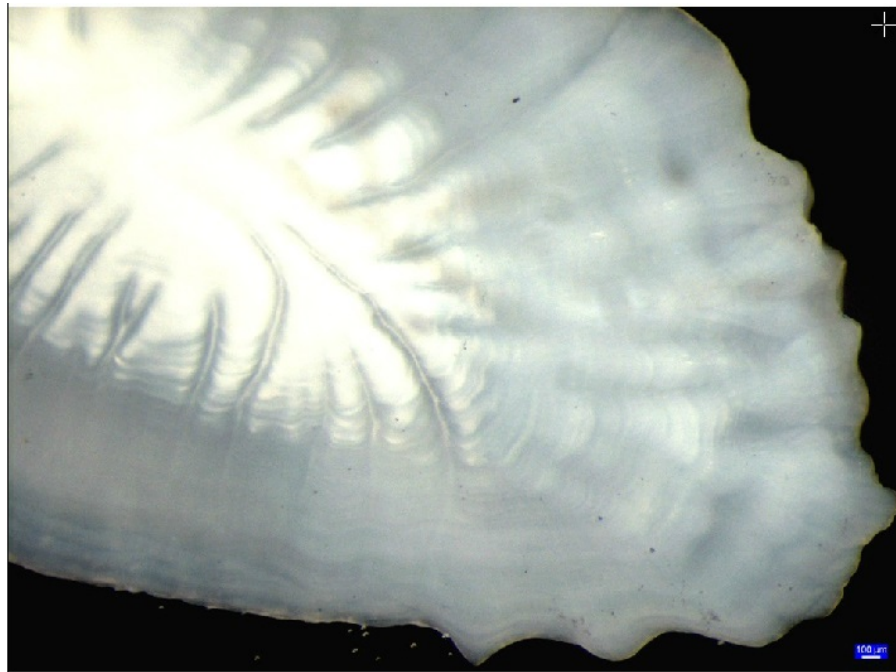
Conclusion

For the *T. mediterraneus* the main source of bias in the ageing analysis are:

- The identification of the first winter; the distance from the core to the first evident ring seems more or less the same of the distance from the first ring to second one.

- The presence of false ring; between the first and second winter ring in correspondence of reaching of the first maturity.

- The overlapping of the transparent ring after the 3th winter ring. Indeed distance between the rings after the first 3 rings decrease as much that it is very difficult to follow the rings around the perimeter of the otolith and then count them.

Regarding the preparation: the whole otolith immersed in a clarification medium (i.e. tap water, seawater) seems give good results. The best otolith orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down with reflected light against a black background.

6.2.3   *Trachurus picturatus* images

12_12_2006_11 (agreement: 70%; CV 31%; modal age: 8; age from readers: 6–15 years; Length: 34 cm; month of capture: December)

Nevertheless the high agreement the age assigned show a big range (6–15 years). During the discussion was underline that the partial vision of the otolith surface (posterior area) not allows to verify the presence of the ring around all otolith.

05_01_2006_15 (agreement: 17%; CV 36%; modal age: 13; age from readers: 6-19 years; Length: 35 cm; month of capture: January)

In this case the agreement is low and the partial vision of the otolith surface (posterior area) not allows verifying the presence of the ring around all otoliths.

JAA_CAN ISL_04 (agreement: 64%; CV 44%; modal age: 2; age from readers: 0-2 years; Length: 18 cm; month of capture: January)

The image was useful to discuss not only for the disagreement of the age, but mostly on the position of the first. Indeed the deposition pattern of the first annuls it is not close at end of the core (withe part of the otolith), but it keep on, up to the deposition of the first winter ring.

COISPA_4976 (agreement: 83%; CV 234%; modal age: 0; age from readers: 0-1 years; Length: 11 cm; month of capture: July)

In this case it was discussed none only the age, but mostly the nature of the edge (translucent, opaque).

JAA_CAN ISL_15 (agreement: 25%; CV 54%; modal age: 3; age from readers: 1-7 years; Length: 28 cm; month of capture: April)

Also in this case was discussed the deposition pattern of the first annuls.

Conclusion

For the *T. picturatus* the main source of bias in the ageing analysis are:

- The identification of the first winter; the deposition pattern of the first annulus it is not close at end of the core (withe part of the otolith), but it keep on up to the deposition of the first winter ring.

- The winter rings are not pronounced probably due to the thickness of the otolith.
- The overlapping of the transparent ring after the 2th winter ring. Indeed distance between the rings after the first two rings decrease as much that it is very difficult to follow the rings around the perimeter of the otolith and then count them.

Regarding the preparation the whole otolith immersed in a clarification medium (i.e. tap water, seawater) seems give good results. The best otolith orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down with reflected light against a black background.

7 Update age reading protocols for each species (ToR d)

7.1 Horse mackerel (*Trachurus trachurus*)

7.1.1 Otolith sampling

This group recommend for each fish to sample both sagittal otoliths (left and right otoliths). For other information on sampling and otolith preparation (see the Annex 1: Review of procedures for otolith preparation and analysis).

7.1.2 Diagram of otolith interpretation

The following (Figure 7.1) is a schematic interpretation of the growth development of *Trachurus trachurus* from its birthday on 1st January following it through to age. The translucent zones are used to determine the age, and towards the end of the year the translucent zone is developing, but should not be counted as a fully developed ring until the 1st January.

Semesters	Edge of otolith	Age
1	Opaque edge (on first quarter) Translucent edge New opaque edge (on May or June)	**N+1** **N** **N**
2	Opaque edge Translucent edge (on November or December)	**N** **N-1**

Figure 7.1: Schematic interpretation of the growth development of the annual zones over the course of a year. The arrows indicate the development of growth rings (translucent and opaque zones) during the year.

An annulus is characterized by the brightest contrast between the preceding translucent and the subsequent opaque zone deposited in the following year. An annulus should be traceable on the whole otolith or the slice, with the exception of the dorso-medial surface of the rostrum. In a section, problems may arise in the area of the *sulcus acusticus* and the dorso-medial direction on the medial side.

7.1.3 Important Guidelines to follow when interpreting the age

- Preference of source of light: the readers use preferably reflected light and black background particularly when image processing is used

- Magnification: the same magnification is recommended to compare the size of growth rings between some otoliths because the widths of consecutive annual growth zones should decrease with increasing age. Be careful with the magnification when reading by stereomicroscope, as a high magnification can cause overestimation of the age with the mistake between false rings and annulii. To identify a lot of annuli on the edge for the older fish, it is possible to zoom in the edge area only.

- Image characteristics: the readers recommend to use only calibrated images (with bar of calibration, pre-treatments of images could induce bias due to different size of otoliths; Figure 7.2) and to see the entire slice or the entire whole otolith to follow the annulus around the whole otolith (for only the first four annulii) or the annulus of each side of the sulcus. Both whole otoliths should be presents on the image. Moreover, the interpretation area of otolith must be clean and without problems due to the preparation (bubble in resin).

Pre treatement with image software

Figure 7.2: Example of the pretreatments of images could induce bias due to different size of otoliths.

- First four growth annuli: on whole otolith, it is possible to follow them all around the nucleus. On slice, it is possible to follow them on each side of the sulcus.

- Relevant data of the otolith interpretation (size, distance of annulus...) from the fish sampled at the same time should be available in case of doubt when assigning fish age.

7.1.4 Observation of whole otolith or slice

Whole otoliths are analysed in a binocular microscope with reflected light against a black background. The best orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down (Figure 7.3). In this way the dark rings could be counted in the posterior area as translucent growth rings. *Rostrum* region is used as the confirmation area.

Figure 7.3: Whole otolith of *Trachurus trachurus* with the main reading axis (red line).

Sliced otoliths are analysed in a binocular microscope. The best otolith orientation for the analysis is from the core area and along each side of *sulcus acusticus* down (Figure 7.4). In this way the dark rings could be counted as translucent growth rings

Figure 7.4: Slice otolith of *Trachurus trachurus* with the main reading axis along each side of *sulcus* (red line).

7.1.5 Quality of images

The readers recommend to use only calibrated images and to see the entire slice or the entire whole otolith to follow the annulus around the whole otolith (for only the first four *annulii*) or the annulus of each side of the sulcus. Both whole otoliths should be presents on the image. The examples of some images with the acceptable quality are presented in Figure 7.5.

Figure 7.5: Calibrated images examples of otolith slices of *Trachurus trachurus* with the acceptable quality because there are good contrast and brightness. Moreover, the slides are realized through the *nucleus*.

In contrary, some examples of slice images without the acceptable quality are presented in the Figure 7.6.

Figure 7.6: Calibrated images examples of otolith slices of *Trachurus trachurus* without the acceptable quality because the slice was not in the nucleus (A.), the entire slice otolith was not observable (B.), there is too brightness (C.) and the preparation is too dirty (D.).

7.2 Mediterranean horse mackerel (*Trachurus mediterraneus*)

7.2.1 Introduction

Mediterranean horse mackerel, *Trachurus mediterraneus* (Steindachner, 1758), is distributed throughout the Mediterranean, the Black Sea, and the northeastern Atlantic (Tortonese, 1975; Whitehead et al., 1986; Fischer et al., 1987). Biological data on Mediterranean horse mackerel are very limited for the entire Mediterranean region (Arneri and Tangerini, 1984; Alegria Hernandez, 1988) and information on ageing accuracy is lacking. By contrast, horse mackerel, which is more abundant in the eastern Atlantic, has attracted much more scientific interest (Belan, 1971; Macer, 1977; Farina Perez, 1983; Kuderskaya, 1983; Arruda, 1984; Kerstan, 1985; Venediktova, 1985; Wysokinski, 1985; Arruda, 1987).

In general, horse mackerel otoliths are very difficult to be read in older fish because they become thick with age (Macer, 1977; Alegria Hernandez, 1984; Kerstan, 1985; Eltink and Kuiter, 1989; Karlou-Riga and Sinis, 1997).

Because of ageing difficulties several otolith exchanges programmes and workshops have taken place in recent years in an attempt to reach agreement on a common way of *annuli*i interpretation (Eltink, 1985; ICSEAF, 1986; Marecos, 1986; Borges, 1989; Eltink and Kuiter, 1989; ICES, 1991, ICES 2012). Although the age interpretation for horse mackerel has been much improved, for Mediterranean horse mackerel the ageing appears to have many problems.

Similarly to *T. trachurus*, the interpretation of the ageing of *T. mediterraneus* otoliths is difficult, mostly for the older specimens where age determination is particularly imprecise. For the otoliths of *T. mediterraneus* there are specific problems to assign the age to younger specimens too and in particular to interpret the first two true *annulus* (Karlou-Riga, 2000), indeed, the characteristic of the detection of a ring around the otolith also on the rostrum zone is not always helpful.

7.2.2 Material and methods

7.2.2.1 Storage

After the extraction, otoliths are washed in order to remove the organic material, than dried and stored in eppendorf

7.2.2.2 Preparation and interpretation

The otoliths are analysed to the binocular microscope with reflected light against a black background. The best otolith orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down (Figure 7.7). In this way the dark rings could be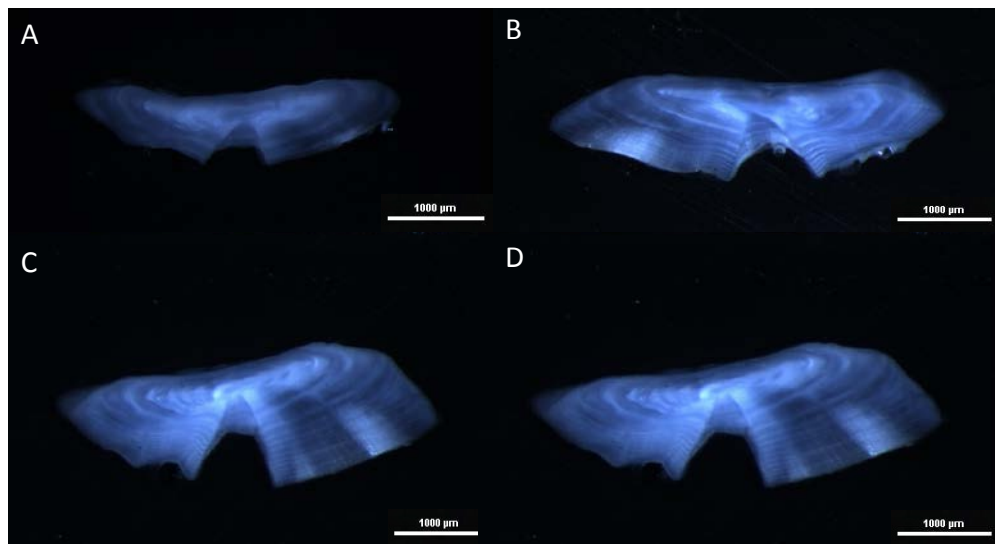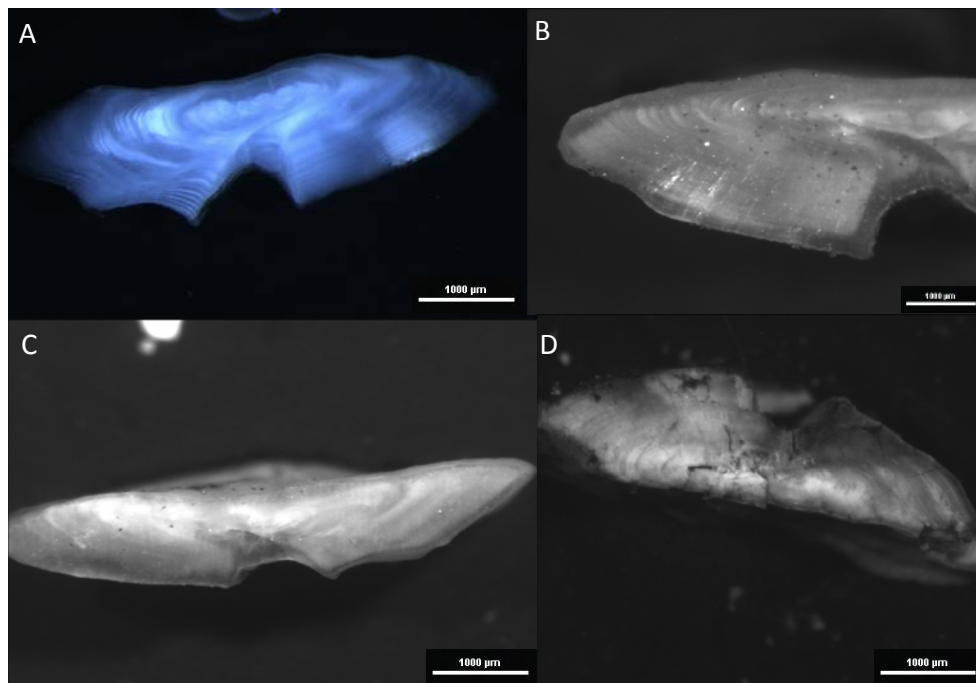 counted in the *posterior* area as translucent growth rings (slow growth). The opaque zone (white – fast growth) plus a dark ring is considered as an annual increment (*annulus*).

Rostrum region is used as the confirmation area.

Distance from the core to each ring is measured in posterior area (post-rostrum) along an axis from to core to the posterior edge of distal face (Figure 7.8).

Otolith is immersed in seawater to be analysed. A solution of glycerine or alcohol 70% can be used too. Usually the otoliths of *T. mediterraneus* need the clarification phase before the age analysis. Permanence time depends on the otolith size (usually

more than one hour). To increase contrast between dark and opaque rings, otoliths can be burnt in an oven for minimum eight hours and 190-200 ºC of temperature, depending the size of the samples, to achieve the best growth rings alternation (Figure 7.9)
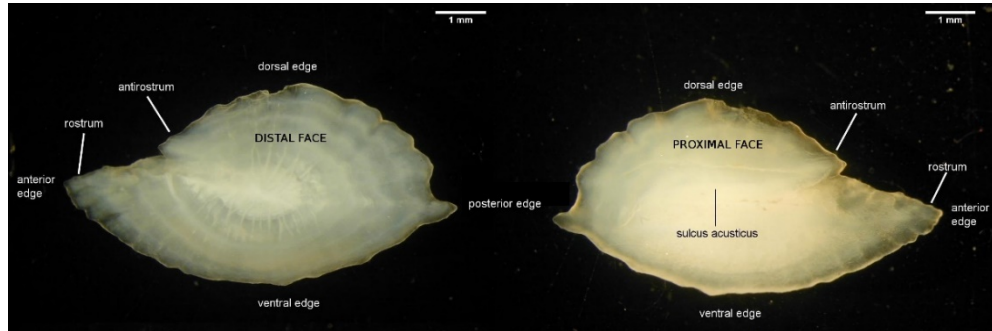

Figure 7.7: Proximal and distal face of *Trachurus mediterraneus* otolith.

Figure 7.8: Post-rostrum area is used to calculate distance from the core to each ring.

Figure 7.9: Burnt otolith of *Trachurus mediterraneus.*

7.2.2.3 Aging

About the ageing criteria, the birthday is set at the 1st of July, according with the spawning period (Vietti et al., 1997; Karlou-Riga et al., 2000). The criteria to age the otoliths reported in Table 7.1 take in count the time of annulus formation (generally transparent ring during winter and spring months; opaque area during summer and autumn months), the capture date, the otolith edge and the spawning period.

In the specimens caught during the second part of the year, if a transparent ring is observed at the edge of the otolith it is not counted as annual ring. Indeed, the opaque edge is not still formed, but the birthday is passed. If a transparent ring is observed at the edge of the otolith at the first semester of the year, the ring on the edge it is not considered as annual ring because the birthday is not reached.

For the specimens with opaque edge caught in the first part of the year the age correspond to the number of the transparent rings, though the transparent ring at the edge is not still formed. For the specimens caught in the second part of the year with the opaque edge, the age correspond to the transparent rings (Figure 7.10)

Table 7.1: The age interpretation criteria on the otoliths to age *Trachurus mediterraneus.* **Birth date: 1st July. N is the number of the transparent rings.**

CATCH DATE	OTOLITH EDGE	AGE
1January–30 June	Transparent	N -1
	Opaque	N-1
1July–31 December	Transparent	N-1
	Opaque	N

Figure 7.10: Scheme age for *T. mediterraneus* (Birth date: 1 July).

Although the results of the studies carried out on spawning period and in order to limit biases due to different approach studies, Table 7.2 reports the criteria to age *T. mediterraneus* using 1st January as birth date.

In the specimens caught during the second part of the year, if a transparent ring is observed at the edge of the otolith it is not counted as annual ring. If a transparent ring is observed at the edge of the otolith at the first semester of the year, the ring on the edge is not considered as annual ran.

For the specimens with opaque edge caught in the first part of the year the age correspond to the number of the transparent rings, though the transparent ring at the edge is not still formed. For the specimens caught in the second part of the year with the opaque edge, the age correspond to the transparent rings (Figure 7.11)

Table 7.2: The age interpretation criteria on the otoliths to age *Trachurus mediterraneus*. Birth date: 1st January. N is the number of the transparent rings.

CATCH DATE	OTOLITH EDGE	AGE
1 January–30 June	Transparent	N
	Opaque	N
1July–31 December	Transparent	N-1
	Opaque	N

Figure 7.11: Scheme age for *T. mediterraneus* (Birth date: 1st January).

7.2.2.4 False and growth annuli

As reported in Karlou-Riga (2000), before the first winter ring some false rings are laid down. Indeed, the small specimens (5–8 cm) caught during summer and autumn months, from spring-summer spawning, present a transparent edge (Figure 7.12). This is a false ring probably laid down when the juveniles changed the environment and the diet.

Figure 7.12: The otoliths from small specimens (A 5 cm and B 7.5 cm total length) caught respectively (A) during summer (29/07/2011) and (B) autumn (06/10/2011).

The measures of this otolith are about 2 mm (0.95 mm *radius*) and the trace of this false ring at the similar measurement is visible also in the otoliths of older specimens (Figure 7.13).

Figure 7.13: Specimen of *T. mediterraneus* with 14.5 cm of total length caught in summer (28/07/2011). The open black circle is a false ring the red one the first winter ring.

The first winter true ring is laid down subsequently and specimens with total length around 12–14 cm caught in the winter and the early spring months present on the edge a transparent ring more evident than the one before, with a measurement on the *radius* of about 1.5 mm (whole otolith measure about 3.5 mm) and a false ring close to the edge (Figure 7.14).

Figure 7.14: Otolith of *T. mediterraneus* of specimen with 12.5 cm of total length caught in the early spring (12/05/2011). The open black circle is a false ring the red one the first winter ring.

Sometimes the first true ring appears not exclusively as a single ring. Indeed, Karlou-Riga *et al.* (2000) distinguish four types of otoliths based on the morphology of the first winter ring (Figure 7.15).

Figure 7.15: The specimens (female 29 cm total length caught on the 15/05/2011) with first winter ring as a transparent zone because the false rings are jointed with the first true ring. The red spot represent the winter ring; the red line represent the first winter.

After the first winter ring, other false ring could be laid down during the second year of life (Figure 7.16). This could be the trace of the reaching of first maturity. Indeed Vietti *et al.* (1997) report for the North Adriatic Sea the first maturity at two years old with 15.6 and 16 cm as the smaller mature specimens respectively for male and female.

Figure 7.16: Otolith of a female with 20.5 cm of total length and the gonads in a post reproductive stage caught during early winter (15/10/2011). The open black circle is a false ring the red one the true winter ring.

After the second winter ring, the deposition pattern of winter band (transparent – black one) appear regular with a reduction of its distance (Figure 7.17).

Figure 7.17: Otholith of *T. mediterraneus* male with 35.5 cm caught in the winter (24/03/2011). The open black circles represent the false rings while the red dots represent the true winter rings.

7.3 Blue jack mackerel (*Trachurus picturatus*)

7.3.1 Whole otoliths

The age determination technique for *T. picturatus* utilizes whole otoliths. Annuli are counted preferentially from the *nucleus* to the posterior margin axis (Figure 7.18).

Figure 7.18: Preferred reading axis in the otolith of *T. picturatus*.

Age reading is done by immersing the whole right otolith from each pair in distilled water for 2-3 hours, under a compound microscope with total magnification of 10x. Whole otoliths must not be mounted or embedded prior to the examination because it may be necessary to lift them up and to view them from different angles. Otoliths are observed under reflected light and dark background with *sulcus acusticus* placed downwards, so dark (translucent/late summer-winter ring) and white (opaque/spring-beginning summer ring) rings could be seen in alternate positions. The direction of the light relative to the otolith surface also needs to be varied. The use of transmitted light is not recommended.

7.3.2 *Annulus* identification

It is commonly agreed that one opaque and one translucent zone constitute an annual growth zone (AGZ) in blue jack mackerel otoliths (Isidro, 1990; Jesus, 1992; Pereira, 1993; Vasconcelos *et al*., 2006; Jurado-Ruzafa and Santamaría, 2014; Garcia *et al*., 2015) (Figure 7.19). It is essential that annuli are identified consistently checking for the same criteria in all otoliths.

Figure 7.19: Otoliths of *T. picturatus* corresponding to fish younger than 1 year (Age class 0). Type of edge can be identified (left: opaque; right: translucent). White arrow indicates the translucent ring width (*In* Jurado-Ruzafa and Santamaría, 2014).

In the case of *T. picturatus* off the Canary Islands, periodicity of annuli deposition has been addressed by means of a translucent edge analysis from qualitative (Figure 7.20) and quantitative (Figure 7.21) approaches (Jurado-Ruzafa and Santamaría, 2014).

Figure 7.20: Monthly evolution of the percentage of translucent vs. opaque edges in otoliths of age classes 0, I and II of *T. picturatus* **(***In* **Jurado-Ruzafa and Santamaría, 2014).**

This particular growth pattern is characteristic of the small pelagic fish inhabiting these waters. This oceanic area, unaffected by the African upwelling and the eddy influence, is characterized by a strong stratification of the water column during most of the year that precludes the input of new nutrients into the photic layer. The quasi-permanent seasonal thermocline is only eroded in January-March because of the winter convection resulting from the slight sea-surface cooling. At that time, the nutrient entrance into the photic layer promotes the so-called "late winter bloom". During the winter bloom, maximum values of chlorophyll *a* concentration and primary production are reached in surface waters, coinciding with the maximum mixed layer depth and nutrient availability. During this productive period, the enhanced phytoplankton growth is followed by an increase of mesozooplankton biomass (Franchy, 2014). Thus, maximum food availability occurs for small pelagic fish off the Canary Islands, resulting in the period of faster growth, and opaque ring deposition (Lorenzo, 1992).

Figure 7.21: Monthly evolution of the translucent edges width (±SD), for otoliths of age class 0 of *T. picturatus* **(***In* **Jurado-Ruzafa and Santamaría, 2014).**

In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). Especially for the first annulus, AGZ should be traceable on the whole otolith, with the exception of the dorso-medial surface of the rostrum. However, in most cases, it does not occur.

In general, the widths of consecutive AGZ decrease with increasing age. Counting annuli after in specimens older than three (when growth rate decrease) is more difficult because of an overlap each other.

It has been identified a high presence of false rings, mainly in the first annulus. Counting each of these well visible rings will result in an overestimation of the age (Figure 7.22).

Figure 7.22: Black dots indicate false rings in the first annulus of *T. picturatus* otoliths from the Canary Islands. Otolith on the left is 3 years old, and the otolith on the right is one year old.

7.3.3 Reading criteria

General adopted criteria for the otolith increments interpretation of *T. picturatus* are:

- Birth date: 1st January.
- Growth pattern scheme: The age assignment is not only depending on the number of annuli, but also on the edge type related to the catch date and the birth date considered. Based on the translucent edge analysis, the pattern annuli deposition is showed in Figure 7.23.

Quarter	Edge of otolith	Age
1	Translucent Opaque	N N-1
2	Translucent Opaque	N-1*
3-4	Translucent Opaque	N-1

Figure 7.23: Scheme of the growth pattern considered for otolith age assignment for *T. picturatus*. (*) explained in the text

- If fish was caught during the year with an opaque zone on the otolith edge, the age assigned will be equal to the number of rings observed minus one.
- If fish was caught in the first quarter with a translucent ring on the otolith edge, the age assigned will be equal to the number of annual rings observed.

- The otoliths with translucent edge of fish caught in the second quarter of the year (*) have to be examined carefully and assessed by the reader, based on the width of this increment. It has to be determined whether this translucent ring corresponds to the finalization of the annulus of the previous year, or to the new translucent ring of the year.
- If fish was caught in the second semester with translucent otolith edge, the age assigned will be equal to the number of annual rings observed minus 1.

7.3.4 Age determination coherency

As it is explained in the "*Annulus* identification" section, the 1 *annulus* deposition per year is validated for the species off the Canary Islands by means of a translucent edge analysis. In addition, age determination coherency has been assessed through *radii* measurement analysis. The frequency distribution of annuli *radii* (Figure 7.24) is in agreement with the seasonal regularity of the growth pattern presented (Morales-Nin, 1991).

Figure 7.24: Frequency distributions of annuli radii (r_i) for the periodic marks considered as annuli, in otoliths of *T. picturatus* from the Canary Islands (*In* Jurado-Ruzafa and Santamaría, 2014).

Reference measurements for *annuli* are presented in Table 7.3, to always compare specimens to the reference measurements.

Table 7.3: Mean *annuli radii* (r_i) measurements (mm), taken from the *nucleus* to the posterior border axis from *T. picturatus* from the Canary Islands.

	ANNULII					
	i	ii	iii	iv	v	vi
Mean ri	2.42	2.89	3.18	3.42	3.57	3.77
SD	0.18	0.21	0.23	0.22	0.19	-

8   Update otoliths reference collections and a database of otoliths images (ToR e)

8.1   *Trachurus trachurus*: slices

8.2 *Trachurus trachurus*: whole otoliths

Portugal 16.4 cm

Portugal 18.1 cm

Portugal 27.2 cm

Portugal 28.5 cm

8.3 *Trachurus mediterranneus*: whole otoliths

Portugal 32.4 cm

Adriatic Sea 21 cm

Adriatic Sea 35.5 cm

Adriatic Sea 15.5 cm

Adriatic Sea 27.5 cm

Adriatic Sea 7.5 cm

false ring

8.4   *Trachurus picturatus*: whole otoliths

Azores 14 cm

Azores 14 cm

Canary Islands, Age class 1 (catch month: march). TL: 17.0 cm

Canary Islands, Age class 2 (caught month: september; last *annulus* is unfinished). TL: 19.8 cm

Canary Islands, Age class 3 (caught month: july; last *annulus* is unfinished). TL: 24.8 cm

Canary Islands, Age class 4 (caught month: july; last *annulus* is unfinished). TL: 27.2 cm

9 Discuss and propose the most appropriate validation methods of age and growth pattern of otolith, for every species and stocks (ToR f)

The validation methods of age is used to verify that the age estimation correspond to true age with a high accuracy. Validation can be direct or indirect (Campana 2001, Table 9.1).

Table 9.1: Most frequent validation methods (from Campana 2001, *In* ICES, 2013) with modifications. Dark green direct validation (A=annual, D=daily).

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

In the first time, for *Trachurus* species, the position of the first growth ring must be clarified

10   References

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Annex 1: List of participants

NAME	ADDRESS	PHONE/FAX	E-MAIL
Alba Jurado-Ruzafa	Centro Oceanográfico de Canarias Carretera de san Andrés s/n E-38120 Santa Cruz de Tenerife Spain	+34 922 54 94 00	alba.jurado@ca.ieo.e
Andrea Massaro	APLYSIA srl C.I.B.M Fisheries Research Laboratory Viale Nazario Sauro 4 57128 Livorno Italy	+39-346970608	andreamassaro@live.it
Bjôrn Vidar Svendesen	Institute of Marine Biology Havforskningsinstituttet Postboks 1870 Nordnes 5817 Bergen Norway	+47 55 23 85 00	bjoernv@imr.no
Clara Dueñas Liaño	Instituto Español de Oceanografia (IEO) Promontorio San Martín s/n 39004 Santander Cantabria Spain	+34 942291084	clara.duenas@st.ieo.es
Eduardo Lopez Diaz	Instituto Español de Oceanografía Centro Oceanográfico de Vigo Subida Radio Faro 50. 36390 Vigo	+34 986 492 111 phone: +34 986 498 626 fax	eduardo.lopez@vi.ieo.es
Gertrud Delfs	Institute of Sea Fisheries Palmaille 9 22767 Hamburg Germany		gertrud.delfs@ti.bund.de
Inaki Rico Uria	AZTI Herrera Kaia Portualde z/g 20110 Pasaia (Gipuzkoa) Spain		irico@azti.es
Jesus Acosta Garcia	Instituto Español de Oceanografía Centro Oceanografico de Malaga Puerto Pesquero s/n 29640 Fuengirola (Malaga) Spain		jesus.acosta@ma.ieo.es
Kelig Mahe (Chair)	Ifremer 150 Quai Gambetta 62321 Boulogne Cédex France	+33 321 995602	Kelig.Mahe@ifremer.fr
Kirsti Boerve Eriksen	Institute of Marine Biology Havforskningsinstituttet Postboks 1870 Nordnes5817 Bergen	+47 55 23 85 00	kirsti.boerve.eriksen@imr.no

	Norway		
M. Teresa Teresa Garcia Sntanaria	Instituto Español de Oceanografía Centro Oceanográfico de Canarias Carretera de san Andrés s/n E-38120 Santa Cruz de Tenerife Spain	+34 922 54 94 00	teresa.garcia@oceanografia.es
Pedro Torres Cutillas	Instituto Español de Oceanografía Centro Oceanografico de Malaga Puerto Pesquero s/n29640 Fuengirola (Málaga) Spain		pedro.torres@ma.ieo.es
Pierluigi Carbonara (Chair)	COISPA Tecnologia e Ricerca Satzione Sperimentale per lo studio delle risorse del mare via dei Trulli 18/20 70126 Bari - Italy	+39 080 5433596	carbonara@coispa.it
Romain Elleboode	Ifremer150 Quai Gambetta62321 Boulogne Cédex France	+33 321 995600	Romain.Elleboode@ifremer.fr

At the top from left to right: Svendsen Bjôrn Vidar, Pedro Torres Cutillas, Iñaki Rico Uria, Clara Dueñas Liaño, Alba Jurado-Ruzafa & Kirsti Boerve Eriksen. At the bottom from left to right: Andrea Massaro, Pierluigi Carbonara, Pedro Torres Cutillas, Jesús Acosta García, Gertrud Delfs, Teresa García Santamaría, Kélig Mahé & Romain Elleboode.

Annex 2: WKARHOM3 terms of reference for the next meeting

The **Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus, T. mediterraneus* and *T. picturatus*)** (WKAR-HOM3), chaired by Alba Jurado, Spain and Pierluigi Carbonara, Italy, and Kélig Ma-hé, France, will be held in Livorno (Italy), 7–11 May 2018, to:

1) Review information on age determination, otolith exchanges and validation study on these species

2) Clarify the position of the first annulus with the images analysis for three species

3) Evaluate the effect of different schemes of ageing particularly the date of birth for *Trachurus mediterraneus*

4) Continue the guidelines and common ageing criteria;

5) Develop existing reference collections of otoliths;

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

Supporting Information

Priority:	Essential. Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Age data are provided by different countries and are estimated using international ageing criteria. It is necessary to continue to clarify this guideline of age interpretation. Therefore, an appropriate otolith exchange programme will be carried out in 2017 for the purpose of inter-calibration between ageing labs. Results of this otolith exchange will be discussed during WKARHOM3.
Scientific justification:	The aim of the workshop is to identify the current ageing problems between readers and standardize the age reading procedures in order to improve the accuracy and precision in the age reading of this species.
Resource requirements:	No specific resource requirement beyond the need for members to prepare for and participate in the meeting.
Participants:	In view of its relevance to the DCF, and ICES WG, the Workshop try to join international experts on growth, age estimation and scientists involved in assessment in order to progress towards a solution. Participants should announce their intention to participate in the WK no later than two months before the meeting.
Secretariat facilities:	
Financial:	
Linkages to advisory committees:	ACOM, SCICOM
Linkages to other committees or groups:	WGBIOP
Linkages to other organizations:	There is a direct link with the EU DCF.

Annex 3: Recommendations

RECOMMENDATIONS	ADRESSED TO
1. WKARHOM2 recommends to use the updated guideline by species for the ageing analysis.	WGBIOP, National Ageing Coordinators
2. WKARHOM2 recommends to organize a new ageing workshop for *Trachurus* species in three years to check the use of ageing criteria and the progress in the precision.	WGBIOP, ACOM
3. WKARHOM2 due the lack of validation study and validation ageing coherency recommends to improve this kind of study (i.e. the marginal analysis and taking measuraments between the rings).	WGBIOP
4. WKARHOM2 reccommends to improve the study of spawning on *T. mediterraneus* in the Atlantic to solve the question of birthday for this species.	WKMSMAC2
5. WKARHOM2 recommends to update WebGr and it's server. During the workshop was WebGr stopped functioning, data could not be extracted and it was impossible to work.	WGBIOP, ACOM

Annex 4: Review of procedures for otolith preparation and analysis

Germany: Thünen Institute of Sea Fisheries in Hamburg (vTI-SF)

Sampling and storing of calcified structures

Horse mackerel from commercial catches and surveys are sampled by the Institute of Sea Fisheries within the EU data collection framework. Sampling takes mostly place in the first and the fourth quarter of the year. Usually, of every 1cm length class 10 *sagittae* otolith pairs are taken during the trips by ICES division and stored in paper envelopes. Each envelope is labelled with cruise number, station, area, fish species, length, weight, sex, maturity and catch date.

In the laboratory one otolith is used for slicing whereas the other otolith is stored dry in Eppendorf tubes. After reading the sliced otolith sections are glued on glass plates which are then also stored. Altogether, 800–1000 horse mackerel otoliths are taken and read on a yearly basis by Germany.

Equipment and preparation of calcified structures

Usually, every left otolith is taken for slicing. The right otoliths are stored in Eppendorf tubes in boxes with all haul and fish information (see paragraph above). Each left otolith is prepared by marking the *nucleus* with a pencil (see Irish description for details).

Under a fume hood a rubber casting mould with marks for the otoliths is half filled with epoxy casting resin (GTS polyester–resin (VossChemie, 35 – 40% Styrol) with 6ml MEKP-hardener). Depending on the size of the otoliths up to 50 otoliths can be embedded in one mould. The otoliths are laid onto the resin bed according to their marks. To fix the otoliths this is done before the resin is completely hardened. Then the casting mould is filled completely with resin. After hardening of the resin the block with the embedded otoliths is placed into a device for series cuts (Figure 1).The block is then half automatically cut by a wet abrasive cut-off machine producing 0.5mm thick slices through the nucleus of the otoliths (Figure 2).

Fig, 1: Device for series cuts of otoliths (Picture by ATM GmbH)

Figure 2: Otolith sawing machine used by the Institute of Sea Fisheries

The slide is interpreted under binocular with the black background with baby oil.

Quality control

Germany has one experienced *Trachurus trachurus* age reader and is currently training a second. The experienced age reader has participated in several international otolith validation exchanges and workshops. Detailed manuals of each processing step (sampling on commercial vessels, embedding the otoliths in resin etc.) are available in German language.

Ireland: Marine Institute

Collection and storage of otoliths

Otoliths are collected in the field with associated metadata. The otoliths are washed in water and stored in 25 well plastic trays with a lid until further processing in the laboratory.

Preparation of otoliths prior to mounting:

Each otolith is prepared before mounting by running a pencil along the *sulcus* and identifying the *nucleus*, then the nucleus is marked (Figure 3). Large otoliths need to have each end clipped so that they can fit in each line. In the case where two otoliths were removed from the fish the left hand side one is marked and then mounted.

Fig 3. Left hand side haddock otolith.

Mould preparation

The moulds are prepared by ensuring they are clean, and are then waxed liberally with mould release wax.

Otolith mounting

The otoliths are orientated so that the *sulcus* with the marked *nucleus* is facing upwards. All of the nuclei are arranged so that they are on the centre line of the block. This is done using the X-Y mounting jig and camera (Figure 4) Ensuring that all otoliths are aligned correctly with a line drawn on the monitor. No otoliths should touch each other; only a small gap is allowed between each otolith.

Fig 4. X-Y jig and screen shot on x-y table

Resin preparation

The otoliths are set in resin in two stages; Mould base and top layer:

Mould base:

- 75g of polyester resin Crystic R115 NTP
- 1ml of catalyst
- 1 spoon of black powder pigment

Under fume hood 75g of polyester resin is poured into the paper cup. 1ml of Catalyst is added using a verified pipette. Mixed slowly for approx. one minute (to avoid air bubbles) but thoroughly using a spatula in order to fully distribute the catalyst. one

spoon of black powdered pigment is added and mixed slowly for another minute to evenly distribute pigment throughout the resin. The prepared resin is used immediately. The resin is poured into each mould to create the thin even layer at the bottom. It is left to set to a tacky consistency (1.5 hours in 20°C). This forms the base of the block.

When otoliths are mounted a top layer of resin is prepared and poured making sure the otoliths are fully covered to rim of the mould.

Top layer:

- 120g of resin
- 1.5ml of catalyst
- 1 spoon of black powder pigment

Under fume hood 120g of polyester resin is poured into the paper cup. Mixed slowly for approx. one minute (to avoid air bubbles) but thoroughly using a spatula in order to fully distribute the catalyst. 1.5 spoon of black powdered pigment is added and mixed slowly for another minute to evenly distribute pigment throughout the resin. The mould is left in the fume hood for 24 hours to cure.

Blocks removal

The blocks are removed by tapping the back of the moulds with a rubber mallet.

Each strip is engraved with the mould number and section number i.e. 200A1; 200A2; 200A3. The edges of the blocks are trimmed with a knife so they lay flat on the sectioning machine jig. When cut, the blocks are allowed to dry in a tray and placed in labelled zip lock bags which are stored in archive boxes.

Otolith Sectioning

A Pilses sectioning machine with Fagor Dro NV-10 digital readout is used for the cutting of slides from the prepared resin blocks containing otoliths. See photo of machine below.

The circular cutting blade used in Pilses sectioning machine allows production of sections as thin as 0.50mm. However based on the best code of practice a thickness of 0.60-0.65mm is recommended, which provides better support to otoliths in the resin during the cutting process and is thin enough to provide the required degree of transparency for observation of the growth rings. (Panfili *et al.,* 2002)

The recommended blade is BUEHLER Diamond Wafering Blade; Series 15 LC Diamond; No. 11-4276; 6"Dia x 0.020" (15.2cm x 0.5mm); Arbor size ½" (12.7mm)

The thin slices are mounted onto glass slide by the application of thin layer of histokitt. For horse mackerel otoliths no histokitt is applied on top. Slides are allowed to dry for a minimum of 72 hours.

The sectioned otoliths are subsequently read under a Leica microscope (35x) using reflective light. Prior to reading the clear "Baby oil" is applied to the slide to enhance the rings and aid reading.

Quality control

Ireland has one experienced *Trachurus* age reader and is currently training a second. The senior age reader has participated in several international otolith validation exchanges/workshops.

4.3 Italy: COISPA – CBM

Sampling

In COISPA and CBM Institutes are collected the otoliths of *T. trachurus* and *T. mediterraneus* and the sampling are carried out mostly during two research and monitoring projects: MEDITS trawl survey in the late spring early summer and CAMPBIOL landing monitoring project (Data Collection Framework, EU Reg. 1543/2000, 1639/2001 and 1581/2004) through all year.

The sampling areas are the South Adriatic sea (MEDITS and CAMPBIOL), Northern Tyrrhenian sea (MEDITS and CAMPBIOL), Central-Southern Tyrrhenian sea (MED-

ITS and CAMPBIOL) and Western Ionian sea (CAMPBIOL), following the FAO classification respectively GSA (GFCM-Geographical Sub-Areas) 18, 10 and 19

Storage

After the extraction the otoliths are washed in order to remove the organic material dried and after stored in a coded plastic tube.

Equipment

The whole otoliths are analysed by the binocular microscope (Leica or BELL) with 25x as magnification. The thin slices are analysed by the optical microscope (Nikon E402) with 10x as magnification. The thin section is performed by the Buehler Isomet low speed or Struers minitom cutting machine. The diamond blade mounted on the cutting machine is the Buehler series 15HC n. 11-4244 diameter 100 mm thickness 0.25 mm. Two component epoxy resin Buehle EPO-KWICK (resin and hardener) are used to embed the otoliths and the resin Entellan Merck are used to mount the thin slices on the glass slides.

Preparation and interpretation of *Trachurus trachurus* otoliths

One of the pair of otoliths (usually the left) is placed in immersion in seawater to be clarified before the analysis. The time of the immersion depending of the size of the fish usually for the otoliths of juveniles specimens (< 20 cm of total length) one or two minute, between 20 and 30 cm of total length no more of two hours and for the specimens with total length > 30 cm about 4 hours. The otoliths are analysed by the binocular microscope in seawater (clarification medium) with reflected light against the black background. The orientation for the analysis is with the distal surface up and the proximal surface (*sulcus acusticus*) down. So the dark rings are counted as the translucent growth zone (slow growth). The opaque (white – fast growth) zone plus a dark ring is considered as *annulus*. The ageing of Atlantic horse mackerel are performed considering 1st of January as the birthday according with the spawning period (Abaunza *et al.*, 2003; Carbonara *et al.*, 2012). Moreover the age is assigned in term of 0.5 years following the scheme reported in Table 1. For the specimens caught in the first part of the years if a transparent ring is observed at the edge of the otolith, is counted as annual ring. If a transparent ring is observed at the edge of the otolith at the second semester of the year, it is not considered as annual ring and the age is equal to the number of *annulus* completed (n) plus 0.5 that represent the half part of the years spent. For the specimens with opaque edge caught in the first part of the years the age corresponds to the number of the *annulus* (n) plus 1, because the transparent edge not is still formed. On the contrary for the specimens caught in the second part of the year with the opaque edge the age corresponds to the completed annulus (n) plus 0.5.

Table1 – The interpretation scheme of *Trachurus trachurus* to assign the age. The n is the number of transparent ring excluded the edge; N is the number of transparent ring included the edge.

Date Capture	Otoliths edge	Age
1 Jannuary-30 June	Transparent	N
	Opaque	n+1
1July-31 December	Transparent	N + 0.5
	Opaque	n + 0.5

Moreover for every otoliths are noted the edge quality (opaque or transparent), the measurements from the core to each transparent ring (at the end of the ring) on the *post-rostrum* area (Figure 5) and the *radius* length.

Figure 5: An example where the measurements are taken during the otoliths analysis. The X are the limits of the each ring and the black line is the core and the white line is the limits of the *radius*

In some case the otoliths of bigger specimens that are difficult to read are embed in the epoxy resin and dorsal-ventral thin (about 550-650 μm) sections through the nucleus are made. The thin slices mounted on the glass slide by the resin (Entellan TM) are analysed under the binocular microscope with the reflected light (transparent rings dark and opaque zone white) and the optical microscope with transmitted light (transparent ring appear light and opaque zone darkness).

Preparation and interpretation of *Trachurus mediterraneus* otoliths

To the ageing of *T. mediterraneus* is followed the same protocol of the *T. trachurus* with some modification. Regard the preparation usually the otoliths of Mediterranean horse mackerel don't need the clarification phase before the age analysis except for the bigger specimens (> 30 cm) where the very short (5–10 minutes) permanence in the seawater could be necessary.

About the ageing criteria the birthday are set at 1st of July according with the spawning period (Vietti *et al.*, 1999; Karlou-Riga *et al.*, 2000) and the criteria to ageing the otoliths is reported in the Table 2.

Table 2: The age interpretation criteria to ageing the otoliths of *Trachurus mediterraneus*. The n is the number of transparent ring excluded the edge (*annulus*); N is the number of transparent ring included the edge.

Date Capture	Otoliths edge	Age
1 Jannuary-30 June	Transparent	N + 0.5
	Opaque	n + 0.5
1July-31 December	Transparent	n
	Opaque	N + 1

Quality control

The same structure are read by two readers and are accepted only the coincident estimation. The percentage of agreement and CV are also analysed. The readings of not expert readers are accepted when the percentage of agreement with the expert readers is > 80%.

The measures taken under the microscope (distance from the core to each ring) are intercalibrated with a defined measure.

Norway: Institute of Marine Research

Sampling and storing of calcified structures

The Institute of Marine Research has during the last years been sampling approximately 10-15.000 ton horse mackerel each year through the reference fleet programme (collaboration with fishers). Of these about 300 otoliths are read every year, 30 specimens from each sample. Sampling is conducted in the last quarter of the year.

Both *sagittae* otoliths are collected from each fish. They are washed thoroughly immediately after collection in order to remove the organic material from the surface and subsequently stored dry in envelopes.

Equipment and preparation of calcified structures

Before the reading one of the otoliths is broken transversely across the short axis through the nucleus. The fractured surface of the anterior half of the broken otolith is polished using wet sandpaper, nr. P600. The rostrum is broken off and the polished part is then burnt over a Bunsen burner for a few seconds while constantly in motion. To clarify the ring structure these otoliths are carefully charred until darkish brown (Mølller Christensen, 1964). The treated otolith is mounted in plasticise and brushed with baby oil on the break. The otoliths are read under a stereomicroscope using direct light, preferably an intensive cold-light source. The translucent rings in the burnt otolith are counted in the large ventral lobe near the *sulcus acusticus*.

Quality control

IMR have only had one experienced *Trachurus trachurus* age reader and is currently training a second one, therefore no reading comparisons have been conducted. The experienced age reader has participated in several international otolith validation exchanges/workshops. No manual for age reading is available at IMR.

Portugal: IPIMAR

Sampling and storing of calcified structures

Every year, several fish samples are collected from three zones: North (Matosinhos harbour), Northwest (Peniche harbour) and South (Portimão harbour) for biological analysis. From these, 15 otoliths per length class of 1 cm are kept for lengths below 20 cm (5 per zone), and 30 otoliths for lengths of 20 cm and above (10 per zone). The otoliths are washed immediately after collected in order to remove the organic material from surface and stored dry in envelopes with the information about the specimen (length, sex, etc.).

Equipment and preparation of calcified structures

For fish smaller than 26 cm, the whole otolith is submerged in a 1% thymol solution (made with filtered and sterilized seawater) for +/- 20h before age readings, and are observed immersed in oil with a binocular microscope, with reflect light against a dark background. For fish larger than 27 cm, the otoliths are embedded in a black epoxy resin (Epoxy resin SP106,Sp106 slow hardener, sp 106 epoxy pigment black) positioned in a row in a specially prepared mould. The resin blocks are cut with a high speed diamond grinding disc. All section are collected and glued on a glass microscope slide for age reading with a binocular microscope with reflect light against a dark background.

Quality control

IPIMAR only has one experienced *Trachurus trachurus* age reader. The experienced age reader has participated in a few international otolith validation exchanges/workshops. No manual for age reading is available at IPIMAR.

Portugal: University of the Azores

Sampling and storing calcified structures

Sampling has been carried out under the EU Data Collection Framework (DCF). By 2008 approximately 30 otoliths (5 per 5 cm length-class) were collected on a monthly basis. From 2009, due to changes in EU regulation, sampling started to be made on a quarterly basis.

From each fish, *sagittae* otoliths are collected and washed in water. Both otoliths are then cleaned, dried and stored in plastic containers (usually eppendorf tubes) before preparation. The species FAO code, date of sampling and the specimen serial number are registered on tracing paper. This piece of paper is inserted in each plastic container. This will allow cross-referencing of the sample with appropriate length, sex, maturity stage, date of capture and weight data taken from each fish.

Equipment and preparation of calcified structures

The work done over the past few years come down to the few post-graduated theses developed with the otoliths collected in the Azores waters. Recently, some otoliths have been observed for age determination. The whole otoliths are used and placed in a Petri dish. The otoliths are immersed in water for posterior reading with binocular microscope. The magnifications used depend on the size of the structure.

Romania: National Institute for Marine Research and Development

Collection and storage of otoliths

Otoliths are collected in the field, are washed in water and stored in plastic trays with a lid until further processing in the laboratory; or when the sample reaches frozen the otoliths are collected after he was thawed and they are degreased in alcohol and stored in plastic trays and after reading them they are kept in special binders with labels where is written the date when the specimen was captured, total length, weight, sex and age.

Otoliths analysis

In the last 10 years in Romania the horse mackerel was not a target species because only small quantities are taken annually so we read about 500 otoliths annually 10 for each length class. In the Romanian Black Sea waters in the last five years the captured individuals were not older than three years.

Spain: AZTI

Sampling and storing calcified structures

The AZTI institute samples around 800 horse mackerel otoliths per year, with about 50 otoliths per sample. From each fish, the date of capture, length, weight, sex and maturity stage are recorded. Otoliths are washed immediately after collection in order to remove the organic material from the surface, and stored dry in envelopes with a sample code.

Equipment and preparation of calcified structures

One otolith of the pair is broken through the nucleus and burnt. One of the burnt halves is mounted in black plasticise and submerged in 70% alcohol, together with the other unbroken otolith. Both are read using reflected light. Age is estimated by interpreting and counting growth rings on the otoliths following methodological ageing procedures described in ICES Workshops (ICES, 1999).

Quality control

AZTI has one experienced *Trachurus trachurus* age reader and is currently training a second. The experienced age reader has participated in several international otolith validation exchanges and workshops. There is no reference collection available in AZTI.

Spain: Canary Islands

Sampling and storing of calcified structures

In the Canary Islands, the fishery of small pelagic fish (mackerels, blue jack mackerels, sardines and sardinellas) is carried out by the artisanal purse-seine fleet. Due to the lack of enough fish markets in the islands, landings are produced in several ports and samples are got from distributors that centralize some of this activity in Tenerife, where are discharged plus of 65% of small pelagic caught in the Canarian Archipelago.

Monthly commercial samples are analysed in the laboratory. All specimens are measured for total length (TL, mm) and weighed for total weight (TW, 0.1 g.), after defrost. Sex and maturity stage are determined through macroscopic examination of the gonads according to a five general maturity stage key (I-immature, II-immature or rest, III-maturing, IV-spawning, and V-spent). In addition, the gonads are removed and weighted (GW, 0.1 g).

Sagitta otoliths are removed following the method established by Goñi (1979) (Figure 6), washed, labelled and stored. It is tried to complete 10 specimens by sex and length class (to the lower cm).

Figure 6: Extraction of *sagitta* otoliths from *T. picturatus*.

Equipment and preparation of calcified structures

One of the otoliths (right preferred) is read on a black support, immersed in water for 2-3 h and examined by two readers under a compound microscope Nikon SMZ1500, with reflected light and 10X magnification.

Photos are taken using a digital camera NIKON DS-5M with a Control Unit NIKON DS-U2 (Figure 7). Images are processed by NIS-Elements 2.3, and the software Image Pro is used in order to take the measurements.

Figure 7: Equipment used in the IEO-Canary Islands to take photos of otoliths of *T. picturatus*.

Reference collection and quality control

In line with growth studies, a reference collection of otoliths are being created with all the species present in the samples analysed in the DCF (Data Collection Framework). Age Readings are carried out by two readers and their disagreements are evaluated by a third reading. Only coincident readings are taken into account to calculate growth parameters. Precision is estimated by the method proposed by Eltink *et al.* (2000). Accuracy cannot be evaluated because data from tagging or tank experiments are not available at the moment and it is not possible the determination of the true age of the individuals.

References

Eltink, A., A.W. Newton, C. Morgado, M.T.G. Santamaría and J. Modin. 2000. Guidelines and Tools for Age Reading Comparisons. Internet: http://www.efan.no: 61 pp.

Goñi, R. 1979. Estudio de crecimiento de *Sardina pilchardus* (Walb.) por lectura directa de otolitos y retrocálculo mediante escalimetría de escamas en ejemplares en la costa NW de África entre los 27°N y 29°N. *Tesina de Máster*. Universidad de Navarra: 126 pp.

Spain: IEO in Málaga

Sampling and storing of calcified structures

Every year, 10 individuals of *Trachurus trachurus* are sampled per 1 cm length class and month in Div. 1.1, which corresponds to the Spanish Mediterranean Sea, sampling area GSA01 (northern Alboran Sea).

Since 2003, the same sampling plan is applied to *T. mediterraneus*, but these samples have not been aged yet, as they were not included in the Data Collection Framework. However, in latest years the landings of that species have increased, and the ageing of the sampled individuals will be started as soon as it is agreed a criteria for that species and area.

Calcified structure (otoliths) preparation

Whole Otolith (*Trachurus trachurus* <24 cm): *Sagitta* otoliths are extracted from each sampled fish and stored dry in envelopes. The otoliths are placed in 70% alcohol to be aged under a microscope using a dark background and reflected light.

Broken and burnt otoliths (*Trachurus trachurus* > 23cm): One otolith of the pair is broken transversely across the short axis through the nucleus. The fractured surface of the anterior half of the broken otolith is polished and the burnt carefully until darkish brown. The treated otolith is mounted on plasticine and submerged in 70 % alcohol together with the untreated whole one. Both are aged and compared under a binocular microscope using a dark background and reflected light.

Age is estimated by interpreting and counting growth rings on the otoliths following methodological ageing procedures described in ICES Workshop (ICES, 1999).

Spain: IEO in Santander

Sampling

Stocks and Geographical areas sampled: *Trachurus trachurus* southern stock (ICES Division IXa North); *Trachurus trachurus* Western stock (ICES Subdivisions VIIIc East

and VIIIc West and Div. VIIIb); *Trachurus mediterraneus* distributed in ICES Division VIIIc (Subdivision VIIIc East and VIIIc West) and IXa North.

Calcified structure (otoliths) preparation

Whole otolith (*Trachurus trachurus* and *Trachurus mediterraneus* <25 cm): Otoliths are extracted from each sampled fish and are stored in micro tubes (until 2011 envelopes were used). The otoliths are placed in alcohol and glycerine, to read with a microscope.

Sectioned otoliths (*Trachurus trachurus* and *Trachurus mediterraneus* >25cm): The whole otoliths are embedded in polyester resin (pre-accelerated polyester resin, black colorant and catalyst) in an aluminium mould. The resin blocks containing the embedded otoliths are removed from the moulds and cut into thin sections (0.5 mm) following the dorso-ventral plane of the otolith. The cutting machine is a high speed saw machine that permits to obtain multiple sections (OTO-LABCUT 230F Floor Standing Abrasive Cutting Machine complete with sectioning fixture and diamond wafering blade, Benetec Limited.). The resulting sections are stuck in glass slides, with the proper label (See IEO Manual of otoliths cuts preparation).

Processing and analysis of images

Images processing and analysis is used for validation studies and for exchanges.

- Image capture/analysis software: Image Analysis Software (NIS-Elements-D 3.0).
- Type of camera: NIS-Elements-D 3.0 and NIS Elements BR Version 2.10.
- Format of images: TIFF files (*.tif), JPEG files (*.jpg), Also jp2 (jpeg2000) files, JASC PAINT SHOP PRO (*.psp).

Quality control

Trachurus trachurus: A specific experienced reader ages the otoliths and each otolith is read twice, on two separate occasions. The readings for a given otolith are accepted only if they are the same. When there is a discrepancy between the two readings, a third reading is carried out. Unreadable otoliths are rejected. From 2012 on, two readers will read the otoliths of this species. Methodological ageing procedures described in ICES Workshops are followed (ICES, 1999; 2006). An age length key is elaborated by month, quarter, semester and year. Mean lengths-at-age and the corresponding standard deviations are calculated.

Trachurus mediterraneus: the criteria and interpretation of growth rings in the otoliths of this species is currently being developed.

Annex 5: Detailed results of exchange 2014

Details results of *Trachurus trachurus*

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

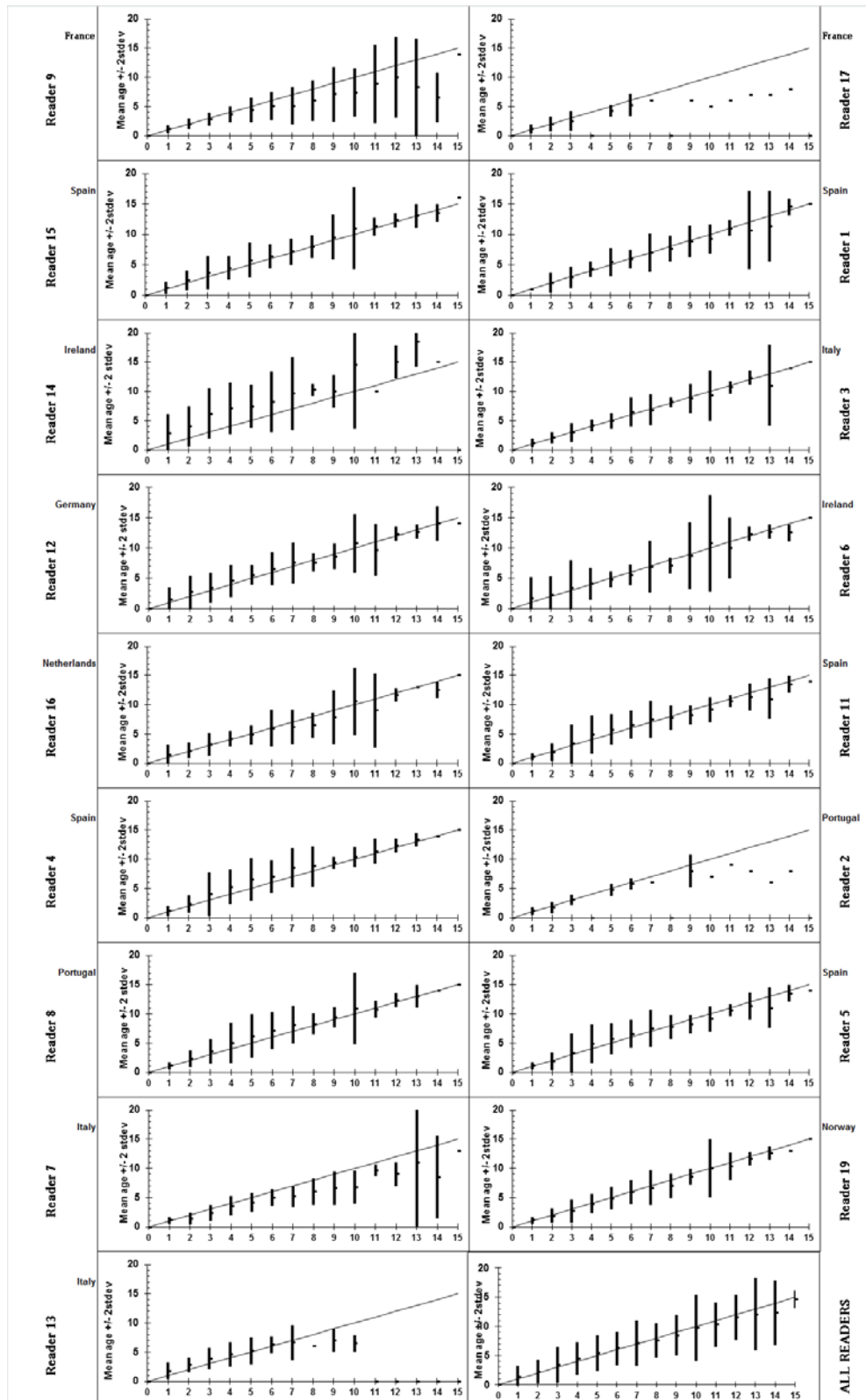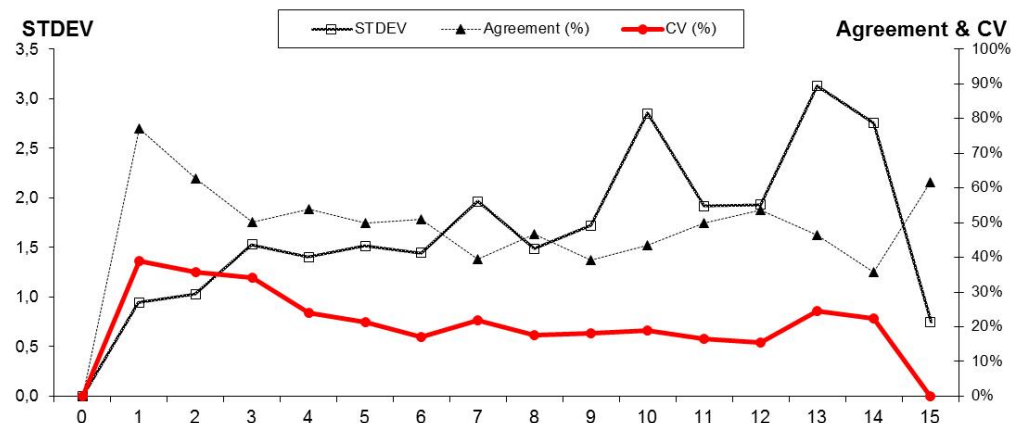| MODAL age	France R9	France R17	Spain R15	Spain R1	Ireland R14	Italy R3	Germany R12	Ireland R6	Netherland R16	Spain R11	Spain R4	Portugal R2	Portugal R8	Spain R5	Italy R7	Norway R19	Italy R13	TOTAL
0																		
1	41	7	45	13	41	27	47	46	43	47	47	9	46	36	10	21	20	546
2	62	6	64	60	46	63	68	68	68	68	68	7	67	67	67	67	26	942
3	50	10	49	42	24	44	50	51	52	52	52	11	46	51	49	51	26	710
4	27	-	27	24	11	24	25	27	27	27	26	-	26	26	27	26	13	363
5	39	4	39	33	26	35	38	39	38	39	39	4	38	39	37	39	14	540
6	20	4	21	20	11	20	21	21	21	21	21	4	21	21	20	21	8	296
7	20	1	20	20	13	20	21	21	21	21	21	1	20	20	20	21	5	287
8	7	-	8	8	4	6	8	8	8	8	8	-	8	8	7	8	1	105
9	10	2	10	9	4	8	10	10	10	10	10	2	10	10	10	10	3	138
10	9	1	9	9	4	9	9	9	9	9	9	1	9	9	8	9	2	124
11	8	1	7	6	1	6	7	8	7	8	8	1	8	8	6	8	-	98
12	3	1	3	3	2	3	3	3	3	3	3	1	3	3	3	3	-	43
13	3	1	3	3	2	3	3	3	3	3	3	1	3	3	3	3	-	43
14	2	1	2	2	1	1	2	2	2	2	2	1	2	2	2	2	-	28
15	1	-	1	1	-	1	1	1	1	1	1	-	1	1	1	1	-	13
Total 0-15	303	39	309	254	191	271	314	318	314	320	319	43	309	306	271	291	118	4290

COEFFICIENT OF VARIATION (CV)

MODAL age	France R9	France R17	Spain R15	Spain R1	Ireland R14	Italy R3	Germany R12	Ireland R6	Netherland R16	Spain R11	Spain R4	Portugal R2	Portugal R8	Spain R5	Italy R7	Norway R19	Italy R13	ALL Readers
0																		
1	30%	33%	39%	0%	61%	32%	71%	106%	62%	31%	38%	30%	26%	26%	29%	27%	41%	39,1%
2	24%	32%	38%	38%	42%	23%	51%	66%	31%	32%	32%	28%	31%	39%	38%	31%	23%	35,7%
3	21%	34%	38%	30%	35%	28%	36%	65%	30%	46%	47%	15%	30%	53%	29%	36%	24%	34,0%
4	19%	-	22%	15%	31%	12%	29%	33%	16%	32%	29%	-	35%	34%	23%	22%	23%	24,1%
5	24%	12%	25%	21%	25%	13%	14%	13%	17%	27%	28%	11%	30%	22%	20%	19%	23%	21,2%
6	24%	18%	16%	13%	32%	20%	21%	16%	26%	19%	20%	9%	22%	18%	15%	18%	11%	17,2%
7	32%	-	15%	23%	32%	19%	22%	31%	24%	19%	20%	-	20%	21%	17%	22%	23%	21,8%
8	29%	-	12%	14%	5%	5%	10%	9%	16%	20%	20%	-	11%	13%	19%	15%	-	17,6%
9	33%	0%	19%	15%	14%	15%	12%	32%	29%	5%	5%	18%	9%	10%	22%	8%	14%	18,0%
10	28%	-	31%	13%	38%	23%	23%	37%	27%	9%	8%	-	28%	12%	21%	25%	11%	19,0%
11	38%	-	7%	6%	-	5%	22%	25%	35%	15%	9%	-	7%	5%	5%	11%	-	16,6%
12	35%	-	5%	30%	9%	5%	5%	5%	5%	5%	5%	-	5%	10%	11%	5%	-	15,5%
13	50%	-	8%	25%	11%	31%	5%	5%	0%	4%	4%	-	8%	16%	64%	5%	-	24,5%
14	33%	-	5%	5%	-	-	10%	6%	6%	0%	0%	-	6%	5%	42%	0%	-	22,4%
15																		
Weighted mean 0-15	25,3%	22,6%	27,7%	23,0%	38,2%	20,5%	36,3%	50,4%	30,0%	28,6%	29,5%	17,3%	26,2%	30,3%	26,3%	24,8%	24,6%	28,7%
RANKING	7	3	10	4	16	2	15	17	13	11	12	1	8	14	9	6	5	

PERCENTAGE AGREEMENT

MODAL age	France R9	France R17	Spain R15	Spain R1	Ireland R14	Italy R3	Germany R12	Ireland R6	Netherland R16	Spain R11	Spain R4	Portugal R2	Portugal R8	Spain R5	Italy R7	Norway R19	Italy R13	ALL
0																		
1	88%	86%	80%	100%	12%	85%	74%	78%	77%	85%	83%	89%	91%	92%	90%	90%	40%	77%
2	82%	67%	56%	60%	13%	78%	44%	85%	65%	79%	78%	71%	70%	69%	31%	64%	31%	63%
3	74%	40%	63%	38%	17%	57%	50%	49%	60%	46%	48%	82%	59%	45%	37%	51%	23%	50%
4	63%	-	67%	67%	9%	75%	28%	52%	67%	44%	42%	-	54%	46%	48%	62%	69%	54%
5	54%	25%	51%	42%	15%	74%	37%	77%	66%	41%	41%	75%	45%	51%	24%	69%	43%	50%
6	45%	50%	57%	70%	27%	60%	48%	57%	71%	43%	43%	75%	52%	52%	20%	52%	50%	51%
7	35%	0%	55%	45%	23%	65%	67%	43%	14%	33%	29%	0%	55%	43%	5%	33%	60%	39%
8	29%	-	63%	88%	0%	83%	38%	25%	13%	75%	75%	-	63%	38%	14%	38%	0%	47%
9	40%	0%	30%	33%	50%	50%	40%	30%	40%	60%	60%	50%	50%	40%	10%	40%	0%	39%
10	11%	0%	33%	67%	0%	44%	44%	44%	67%	56%	56%	0%	78%	44%	0%	56%	0%	50%
11	50%	0%	43%	67%	0%	67%	43%	50%	29%	75%	98%	0%	50%	63%	0%	38%	-	50%
12	67%	0%	67%	33%	0%	67%	67%	67%	67%	67%	67%	0%	67%	67%	0%	67%	-	53%
13	33%	0%	33%	67%	0%	67%	67%	67%	100%	67%	67%	0%	33%	0%	0%	67%	-	47%
14	0%	0%	50%	50%	0%	100%	0%	0%	0%	100%	100%	0%	100%	50%	0%	0%	-	36%
15	0%	-	100%	100%	-	100%	100%	100%	100%	100%	100%	-	100%	0%	0%	100%	-	62%
Weighted mean 0-15	63,4%	43,6%	58,9%	56,3%	14,7%	69,7%	48,7%	63,5%	59,9%	60,0%	59,6%	67,4%	63,4%	56,5%	28,4%	58,1%	37,3%	55,1%
RANKING	5	14	9	12	17	1	13	3	7	6	8	2	4	11	16	10	15	

RELATIVE BIAS

MODAL age	France R9	France R17	Spain R15	Spain R1	Ireland R14	Italy R3	Germany R12	Ireland R6	Netherland R16	Spain R11	Spain R4	Portugal R2	Portugal R8	Spain R5	Italy R7	Norway R19	Italy R13	ALL
0																		
1	0,12	0,14	0,22	0,00	1,76	0,15	0,43	0,65	0,37	0,15	0,19	0,08	0,11	0,09	0,10	0,10	0,75	0,37
2	0,03	0,00	0,42	0,07	2,02	0,06	0,71	0,31	0,12	0,29	0,35	-0,29	0,31	-0,13	-0,66	-0,12	0,81	0,24
3	-0,18	-0,50	0,63	-0,05	3,21	-0,09	0,46	0,47	0,17	0,94	1,02	0,00	0,52	0,24	-0,63	-0,31	0,92	0,36
4	-0,37	-	0,44	0,29	3,09	0,08	0,56	0,07	0,15	1,37	1,27	-	1,00	0,88	-0,41	-0,08	0,62	0,49
5	-0,64	-0,75	0,74	0,45	2,42	-0,09	0,55	-0,18	-0,18	1,51	1,56	-0,25	1,21	0,74	-0,89	-0,10	0,21	0,45
6	-0,95	-0,75	0,38	-0,10	2,18	0,45	0,57	-0,48	-0,05	0,86	1,00	-0,25	1,10	0,57	-1,00	-0,10	0,25	0,24
7	-1,90	-1,00	0,15	-0,05	2,62	-0,20	0,52	-0,10	-0,86	1,29	1,52	-1,00	1,10	0,48	-1,85	-0,33	-0,40	0,10
8	-2,00	-	0,00	0,38	2,25	0,17	-0,38	-0,88	-1,50	0,75	0,75	-	0,25	-0,25	-1,00	-2,00	-	-0,39
9	-1,90	-3,00	0,50	-0,22	1,00	-0,25	-0,40	-0,30	-1,20	0,40	0,40	-1,00	0,40	-0,80	-2,40	-0,40	-2,00	-0,51
10	-2,67	-5,00	1,00	-0,78	4,50	-0,78	0,78	0,56	0,33	0,33	-3,00	0,89	-0,89	-3,25	0,00	-3,50		-0,22
11	-2,13	-5,00	0,29	0,00	-1,00	-0,33	-1,29	-1,00	-2,00	0,75	0,38	-2,00	-0,25	-0,38	-1,33	-0,63	-	-0,66
12	-2,00	-5,00	0,33	-1,33	3,00	0,33	0,33	0,33	-0,33	0,33	0,33	-4,00	0,33	-0,67	-0,33	-0,33	-	-0,44
13	-4,67	-6,00	0,00	-1,67	5,50	-2,00	-0,33	-0,33	0,00	0,33	0,33	-7,00	0,00	-2,00	-2,00	-0,33	-	-0,93
14	-7,50	-6,00	-0,50	0,50	1,00	0,00	-1,50	-1,50	-1,50	0,00	0,00	-	-0,50	-5,50	-1,00		-	-1,64
15	-1,00	-	1,00	0,00		0,00	-1,00	0,00	0,00	0,00	0,00	-	-1,00	-2,00	0,00		-	-0,31
Weighted mean 0-15	-0,67	-1,13	0,44	0,00	2,33	-0,03	0,44	0,14	-0,08	0,74	0,79	-0,65	0,58	0,16	-1,01	-0,20	0,47	0,21
RANKING	12	16	8	1	17	2	7	4	3	13	14	11	10	5	15	6	9	

In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

The coefficient of variation (CV %), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. It appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

Details results of *Trachurus picturatus*

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

MODAL age	Germany Reader 12	Spain Reader 4	France Reader 9	Portugal Reader 8	Portugal Reader 2	Italy Reader 10	Italy Reader 3	Ireland Reader 6	Ireland Reader 14	Spain Reader 1	Spain Reader 5	Italy Reader 7	Spain Reader 11	TOTAL
0	25	25	25	25	24	25	25	25	-	24	25	25	25	298
1	28	27	28	28	17	28	27	28	4	28	27	28	28	326
2	16	16	16	16	14	15	15	16	-	16	16	15	16	187
3	7	7	7	7	7	7	6	7	-	7	6	7	7	82
4	1	1	1	1	-	1	1	1	-	1	1	1	1	11
5	3	3	3	3	1	2	3	3	1	3	3	3	3	34
6	2	3	3	2	-	3	2	3	-	3	3	3	3	30
7	4	5	5	4	-	4	2	5	3	5	5	4	5	51
8	10	12	12	12	-	6	5	12	4	12	12	11	12	120
9	11	13	13	12	-	11	5	13	3	13	13	13	13	133
10	4	6	6	6	-	5	3	6	2	6	6	6	6	62
11	4	5	5	5	-	5	2	5	2	5	5	5	5	53
12	2	2	2	2	-	2	2	2	1	2	2	2	2	23
13	3	4	4	4	-	4	2	4	1	4	4	4	4	42
14	1	2	2	2	-	2	1	2	-	2	2	2	2	20
15	-	1	1	1	-	1	-	1	-	1	1	1	1	-
Total 0-15	121	132	133	130	63	121	101	133	21	132	131	130	133	1481

COEFFICIENT OF VARIATION (CV)

MODAL age	Germany Reader 12	Spain Reader 4	France Reader 9	Portugal Reader 8	Portugal Reader 2	Italy Reader 10	Italy Reader 3	Ireland Reader 6	Ireland Reader 14	Spain Reader 1	Spain Reader 5	Italy Reader 7	Spain Reader 11	ALL Readers
0	125%	276%	106%	234%	0%	0%	149%	115%	-	270%	115%	0%	346%	191,5%
1	40%	51%	48%	46%	94%	118%	43%	51%	7%	57%	92%	69%	47%	64,9%
2	38%	37%	68%	32%	30%	135%	19%	12%	-	51%	63%	56%	37%	46,2%
3	28%	38%	41%	35%	28%	79%	21%	21%	-	19%	35%	44%	37%	36,8%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	44%	36%	60%	29%	-	35%	27%	54%	-	20%	20%	43%	44%	32,6%
6	20%	18%	0%	31%	-	22%	11%	16%	-	0%	22%	9%	11%	29,8%
7	9%	34%	18%	22%	-	35%	0%	32%	25%	18%	27%	24%	30%	33,8%
8	12%	22%	18%	19%	-	14%	20%	20%	11%	19%	19%	24%	28%	26,7%
9	17%	21%	20%	20%	-	27%	6%	26%	8%	21%	34%	23%	20%	28,7%
10	5%	13%	14%	16%	-	24%	33%	16%	9%	13%	5%	12%	15%	23,9%
11	17%	8%	25%	5%	-	27%	8%	19%	18%	15%	9%	18%	8%	25,9%
12	28%	0%	16%	5%	-	8%	9%	20%	-	8%	6%	0%	0%	25,3%
13	10%	0%	11%	6%	-	12%	16%	13%	-	25%	8%	12%	0%	23,9%
14	-	0%	14%	0%	-	9%	-	6%	-	8%	6%	8%	6%	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean 0-15	47,3%	76,8%	48,4%	67,2%	35,1%	56,8%	56,4%	44,3%	10,8%	75,6%	58,3%	31,7%	89,2%	69,0%
RANKING	5	12	6	10	3	8	7	4	1	11	9	2	13	

PERCENTAGE AGREEMENT

MODAL age	Germany Reader 12	Spain Reader 4	France Reader 9	Portugal Reader 8	Portugal Reader 2	Italy Reader 10	Italy Reader 3	Ireland Reader 6	Ireland Reader 14	Spain Reader 1	Spain Reader 5	Italy Reader 7	Spain Reader 11	ALL
0	60%	88%	52%	84%	100%	100%	68%	56%	-	88%	56%	100%	92%	79%
1	61%	56%	57%	32%	53%	43%	37%	82%	0%	68%	56%	68%	71%	56%
2	56%	81%	31%	44%	64%	7%	80%	94%	-	56%	38%	20%	69%	53%
3	57%	57%	29%	43%	43%	29%	67%	57%	-	71%	50%	14%	43%	46%
4	0%	0%	100%	0%	-	100%	0%	0%	-	0%	0%	100%	0%	27%
5	33%	67%	33%	33%	0%	50%	67%	33%	0%	33%	67%	67%	33%	44%
6	50%	0%	100%	0%	-	67%	50%	0%	-	100%	33%	67%	0%	43%
7	0%	40%	20%	0%	-	50%	0%	0%	0%	20%	40%	75%	40%	25%
8	80%	33%	50%	17%	-	17%	40%	25%	0%	33%	33%	36%	42%	36%
9	45%	31%	23%	8%	-	0%	60%	38%	0%	15%	46%	31%	38%	29%
10	75%	17%	17%	0%	-	20%	33%	0%	0%	67%	50%	0%	50%	27%
11	25%	80%	0%	40%	-	0%	0%	20%	0%	0%	20%	0%	80%	25%
12	50%	100%	0%	0%	-	0%	0%	50%	0%	0%	50%	0%	100%	30%
13	33%	100%	0%	50%	-	0%	0%	50%	0%	0%	0%	0%	100%	31%
14	0%	100%	0%	100%	-	0%	0%	0%	-	0%	0%	0%	100%	30%
15	-	100%	0%	0%	-	0%	-	100%	-	0%	0%	0%	100%	-
Weighted mean 0-15	54,5%	60,6%	39,1%	38,5%	71,4%	39,7%	51,5%	52,6%	0,0%	52,3%	44,3%	49,2%	64,7%	50,0%
RANKING	4	3	11	12	1	10	7	5	13	6	9	8	2	

RELATIVE BIAS

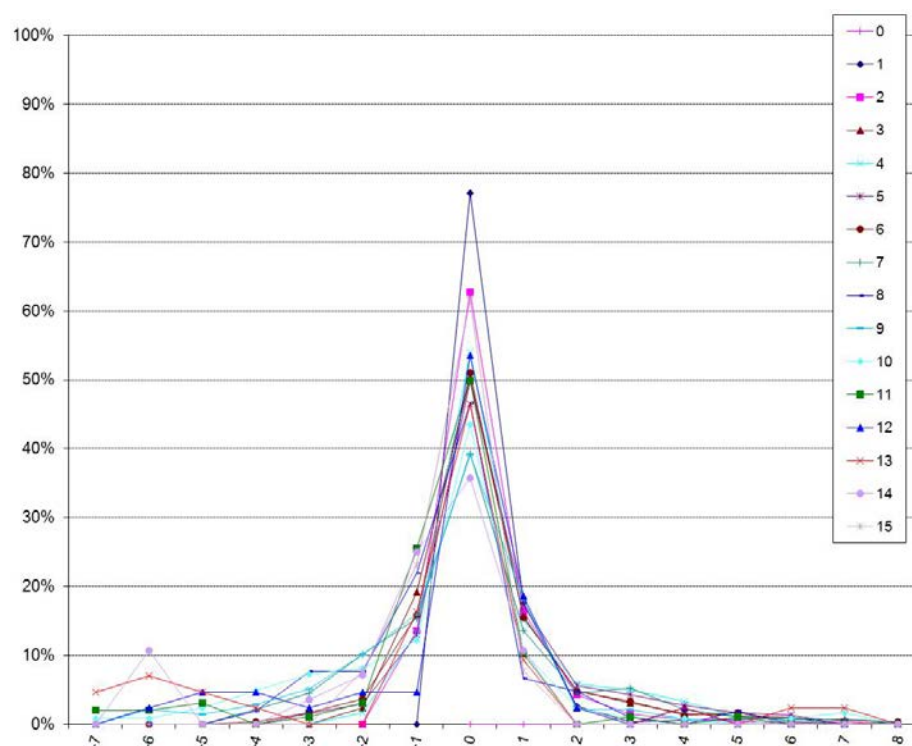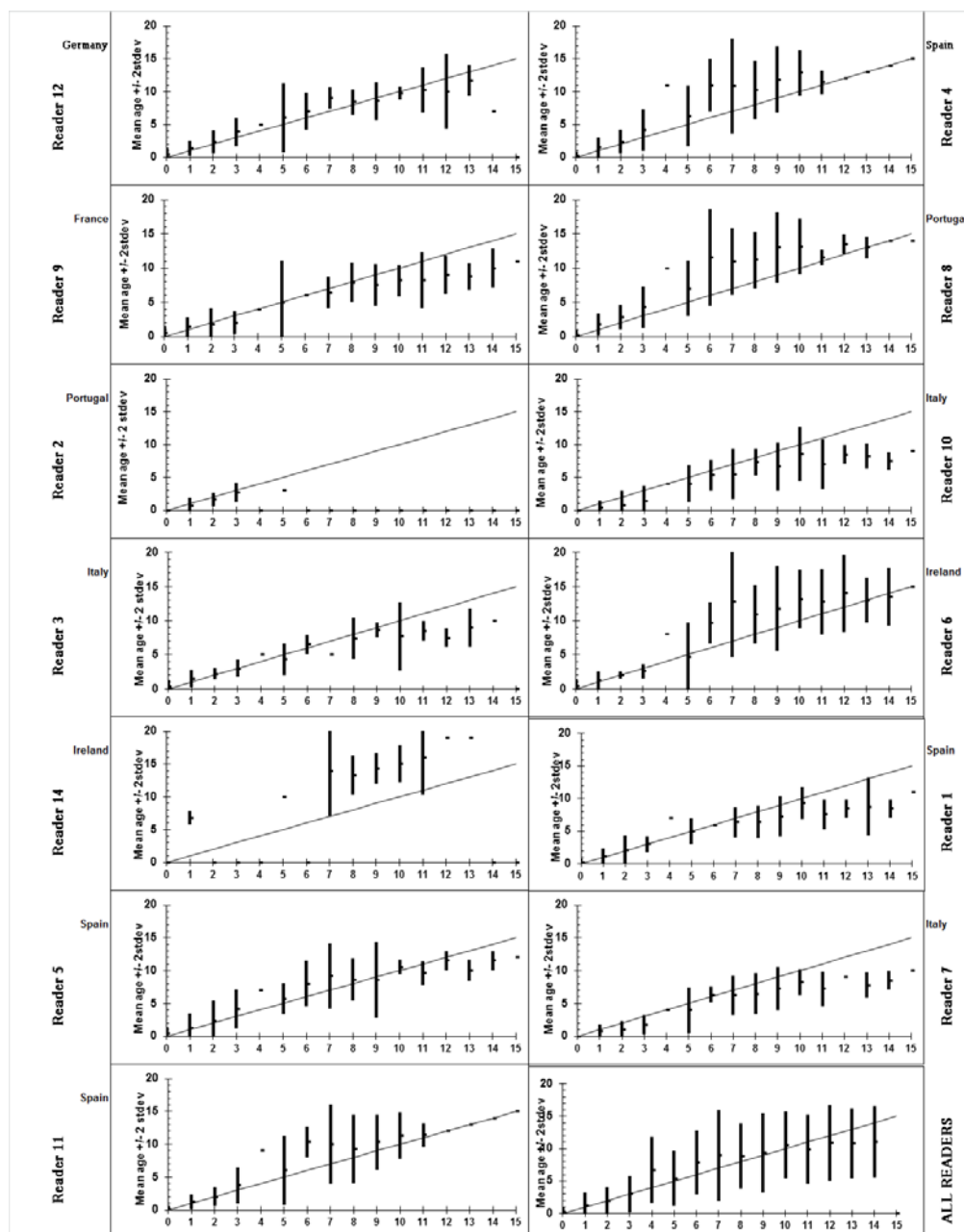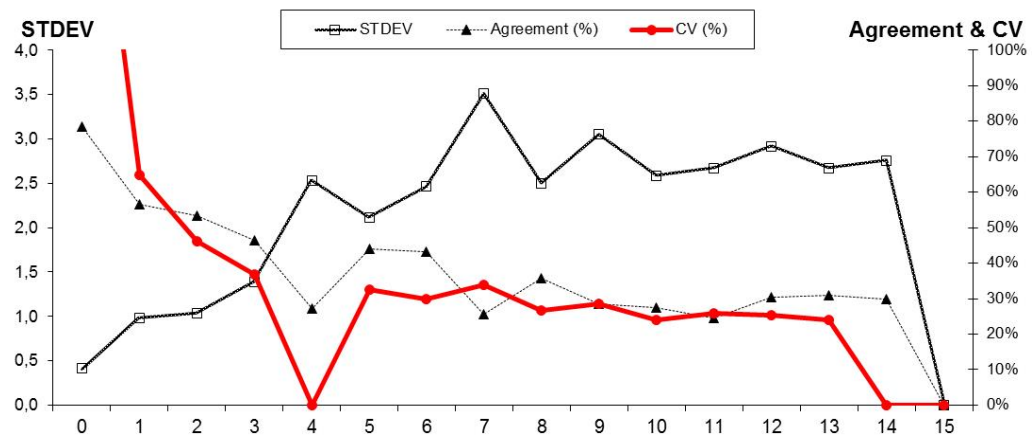| MODAL age	Germany Reader 12	Spain Reader 4	France Reader 9	Portugal Reader 8	Portugal Reader 2	Italy Reader 10	Italy Reader 3	Ireland Reader 6	Ireland Reader 14	Spain Reader 1	Spain Reader 5	Italy Reader 7	Spain Reader 11	ALL
0	0,40	0,12	0,48	0,16	0,00	0,00	0,32	0,44	-	0,13	0,44	0,00	0,08	0,21
1	0,43	0,48	0,43	0,75	-0,35	-0,57	0,48	0,29	5,75	0,14	0,22	-0,25	0,25	0,28
2	0,31	0,38	-0,25	0,81	-0,36	-1,20	0,20	0,06	-	0,13	0,38	-0,93	0,00	-0,03
3	0,86	1,14	-1,00	1,29	-0,29	-1,57	0,00	-0,43	-	0,00	1,17	-1,29	0,71	0,04
4	1,00	7,00	0,00	6,00	-	0,00	1,00	4,00	-	3,00	3,00	0,00	5,00	2,73
5	1,00	1,33	0,00	2,00	-2,00	-1,00	-0,67	-0,33	5,00	0,00	0,67	-1,00	1,00	0,38
6	1,00	5,00	0,00	5,50	-	-0,67	0,50	3,67	-	0,00	2,00	0,33	4,33	1,93
7	2,00	3,80	-0,60	4,00	-	-1,50	-2,00	5,80	7,00	-0,60	2,20	-0,75	3,00	1,96
8	0,40	2,25	-0,08	3,17	-	-0,67	-0,60	2,92	5,25	-1,58	0,58	-1,55	1,33	0,87
9	-0,45	2,85	-1,46	4,00	-	-2,36	-0,40	2,77	5,33	-1,77	-0,46	-1,77	1,31	0,38
10	-0,25	2,83	-1,83	3,17	-	-1,40	-2,33	3,17	5,00	-0,67	0,50	-1,83	1,33	0,56
11	-0,75	0,40	-2,80	0,60	-	-4,00	-2,50	1,80	5,00	-3,40	-1,40	-3,80	0,40	-1,11
12	-2,00	0,00	-3,00	1,50	-	-3,50	-4,50	2,00	7,00	-3,50	-0,50	-3,00	0,00	-1,13
13	-1,33	0,00	-4,25	0,00	-	-4,75	-4,00	0,00	6,00	-4,25	-3,00	-5,25	0,00	-2,19
14	-7,00	0,00	-4,00	0,00	-	-6,50	-4,00	-0,50	-	-5,50	-2,50	-5,50	0,00	-3,00
15	-	0,00	-4,00	-1,00	-	-6,00	-	0,00	-	-4,00	-3,00	-5,00	0,00	-
Weighted mean 0-15	0,22	1,20	-0,53	1,51	-0,24	-1,30	-0,18	1,22	5,67	-0,70	0,21	-1,14	0,70	0,21
RANKING	3	9	5	12	4	11	1	10	13	7	2	8	6	

In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

The coefficient of variation (CV %), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. It appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

Details results of Trachurus mediterraneus

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

MODAL age	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	France Reader 9	Italy Reader 10	Germany Reader 12	Ireland Reader 6	Portugal Reader 2	Portugal Reader 8	Spain Reader 4	Spain Reader 5	Italy Reader 7	Spain Reader 11	Italy Reader 13	TOTAL
0	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	59
1	10	10	10	7	10	8	10	10	10	10	10	10	10	10	9	144
2	12	10	12	11	12	10	12	12	12	12	12	12	12	12	12	175
3	19	17	20	18	19	19	19	20	20	20	20	20	20	20	20	291
4	12	11	12	12	11	10	12	12	12	9	12	12	12	12	12	173
5	13	11	13	11	12	9	13	13	13	12	13	13	13	13	13	185
6	13	13	13	12	13	11	13	13	13	13	13	13	13	13	13	192
7	6	6	6	4	6	6	5	6	5	6	6	6	6	6	6	86
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	3	3	3	2	2	3	3	3	3	3	3	3	3	3	3	43
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	14
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total 0-15	94	87	95	83	89	82	93	95	94	91	95	95	95	95	94	1377

COEFFICIENT OF VARIATION (CV)

MODAL age	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	France Reader 9	Italy Reader 10	Germany Reader 12	Ireland Reader 6	Portugal Reader 2	Portugal Reader 8	Spain Reader 4	Spain Reader 5	Italy Reader 7	Spain Reader 11	Italy Reader 13	ALL Readers
0	0%	0%	0%	0%	87%	0%	0%	115%	0%	0%	0%	0%	0%	0%	0%	215,3%
1	50%	0%	35%	55%	72%	119%	92%	61%	66%	35%	29%	73%	82%	29%	34%	59,5%
2	28%	37%	27%	33%	60%	72%	59%	35%	32%	35%	27%	14%	37%	27%	25%	43,9%
3	33%	36%	48%	25%	26%	52%	33%	32%	22%	58%	50%	54%	31%	46%	49%	33,4%
4	32%	31%	32%	29%	24%	35%	31%	18%	23%	33%	17%	37%	29%	17%	25%	31,8%
5	13%	11%	31%	51%	26%	43%	36%	23%	58%	13%	10%	16%	42%	23%	26%	36,5%
6	0%	19%	19%	37%	26%	32%	19%	24%	36%	11%	15%	15%	29%	10%	29%	23,6%
7	6%	15%	17%	74%	35%	35%	7%	16%	31%	19%	11%	9%	36%	7%	24%	36,9%
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	10%	11%	17%	40%	90%	39%	18%	28%	48%	6%	0%	19%	108%	6%	43%	30,3%
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	6%	-	-	-	-	-	6%	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean 0-15	22,1%	20,8%	29,7%	35,4%	39,2%	49,7%	36,8%	32,9%	34,8%	29,2%	23,2%	30,8%	38,8%	23,3%	30,5%	43,8%
RANKING	2	1	6	11	14	15	12	9	10	5	3	8	13	4	7	

PERCENTAGE AGREEMENT

MODAL age	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	France Reader 9	Italy Reader 10	Germany Reader 12	Ireland Reader 6	Portugal Reader 2	Portugal Reader 8	Spain Reader 4	Spain Reader 5	Italy Reader 7	Spain Reader 11	Italy Reader 13	ALL
0	100%	100%	0%	100%	33%	100%	0%	50%	100%	100%	100%	100%	100%	100%	100%	80%
1	70%	100%	0%	57%	80%	38%	40%	30%	40%	80%	90%	90%	50%	90%	44%	60%
2	75%	30%	0%	45%	33%	30%	50%	17%	58%	58%	67%	92%	50%	67%	75%	50%
3	63%	53%	0%	72%	58%	21%	32%	20%	75%	40%	55%	35%	65%	60%	40%	46%
4	58%	55%	17%	33%	45%	20%	58%	67%	33%	22%	42%	25%	25%	50%	33%	39%
5	85%	64%	8%	9%	8%	33%	23%	62%	8%	58%	77%	46%	23%	69%	38%	41%
6	100%	69%	23%	25%	15%	36%	38%	15%	38%	77%	54%	62%	38%	77%	15%	46%
7	83%	33%	0%	0%	0%	17%	60%	50%	0%	67%	50%	67%	17%	67%	50%	38%
8	0%	0%	0%	100%	0%	0%	0%	0%	0%	100%	100%	0%	0%	100%	100%	33%
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	33%	33%	33%	0%	0%	0%	33%	0%	0%	67%	100%	67%	33%	67%	67%	37%
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	100%	100%	0%	100%	-	0%	100%	0%	0%	0%	100%	100%	100%	100%	100%	64%
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean 0-15	74,5%	59,8%	7,4%	43,4%	36,0%	29,3%	38,7%	33,7%	42,6%	58,2%	65,3%	57,9%	44,2%	69,5%	45,7%	47,2%
RANKING	1	4	15	9	12	14	11	13	10	5	3	6	8	2	7	

RELATIVE BIAS

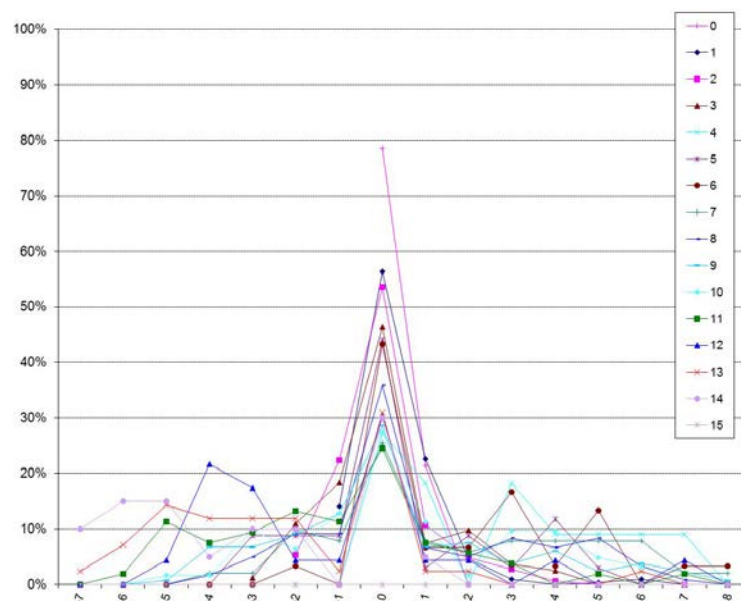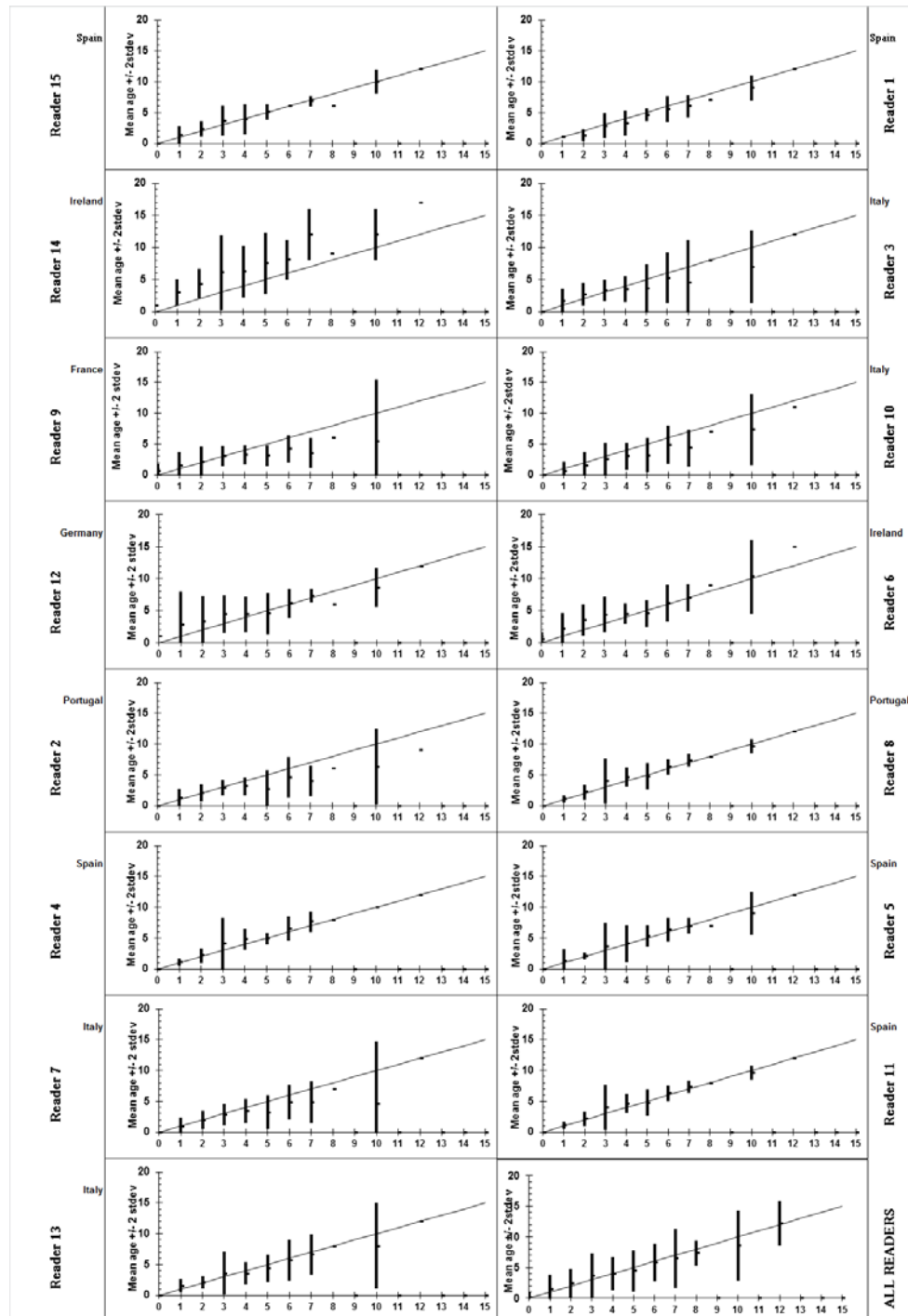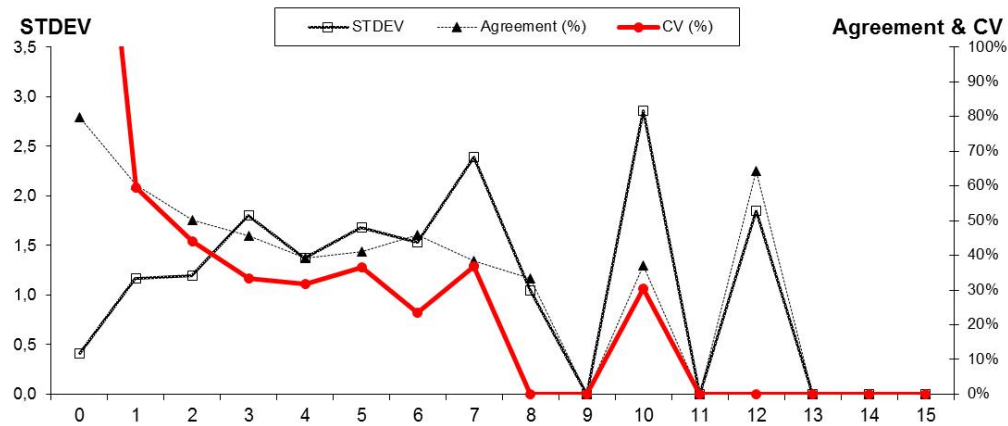| MODAL age	Spain Reader 15	Spain Reader 1	Ireland Reader 14	Italy Reader 3	France Reader 9	Italy Reader 10	Germany Reader 12	Ireland Reader 6	Portugal Reader 2	Portugal Reader 8	Spain Reader 4	Spain Reader 5	Italy Reader 7	Spain Reader 11	Italy Reader 13	ALL
0	0,00	0,00	1,00	0,00	0,67	0,00	1,00	0,50	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,20
1	0,40	0,00	2,00	0,71	0,50	-0,38	1,80	1,10	0,20	0,20	0,10	0,30	-0,10	0,10	0,56	0,51
2	0,33	-0,70	2,33	0,73	0,08	-0,50	1,33	1,58	0,08	-0,08	0,17	0,08	0,00	0,17	0,08	0,40
3	0,63	-0,18	3,10	0,28	0,05	-0,47	1,47	1,40	-0,10	0,70	1,15	0,60	-0,15	1,00	0,55	0,68
4	-0,08	-0,73	2,25	-0,50	-0,73	-1,00	0,42	0,50	-0,83	-0,11	0,83	0,08	-0,58	0,67	-0,42	0,01
5	0,08	-0,36	2,54	-1,36	-1,83	-1,78	-0,46	-0,46	-2,31	-0,25	-0,08	0,31	-1,77	-0,23	-0,62	-0,54
6	0,00	-0,38	2,08	-0,75	-1,69	-1,09	0,15	0,15	-1,38	0,15	0,54	0,38	-1,15	0,31	-0,31	-0,19
7	-0,17	-1,00	5,00	-2,50	-3,50	-2,67	0,40	0,00	-3,00	0,33	0,67	0,00	-2,17	0,33	-0,33	-0,51
8	-2,00	-1,00	1,00	0,00	-2,00	-1,00	-2,00	1,00	-2,00	0,00	0,00	-1,00	-1,00	0,00	0,00	-0,67
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0,00	-1,00	2,00	-3,00	-4,50	-2,67	-1,33	0,33	-3,67	0,33	0,00	-1,00	-5,33	-0,33	-2,00	-1,37
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	0,00	0,00	5,00	0,00	-	-1,00	0,00	3,00	-3,00	-1,00	0,00	0,00	0,00	0,00	0,00	0,21
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weighted mean 0-15	0,18	-0,43	2,56	-0,34	-0,84	-0,99	0,68	0,71	-0,94	0,16	0,48	0,23	-0,83	0,35	-0,09	0,08
RANKING	3	7	15	5	12	14	9	10	13	2	8	4	11	6	1	

In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

The coefficient of variation (CV %), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. It appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.