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Report of the second Workshop on Age Reading of Red Mullet and Striped Red Mullet

2 – 6 July 2012

Boulogne-sur-Mer, France



ICES

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Summary

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) and the Planning Group of Mediterranean (PGMED) meetings in 2011 (ICES, 2011a) recommended an otoliths and scales exchange for *Mullus* species from Atlantic and Mediterranean sea. Workshop on Age reading red mullet (*Mullus barbatus*) and striped red mullet (*Mullus surmuletus*) (WKACM2, chair : K. Mahé, France) has been held in Boulogne sur Mer (France) from 2 to 6 July 2012. Five countries took part in this exchange (Spain, Greece, Cyprus, Italy, France, for a total of 8 participants) during 2011, and 3 countries (Spain, Italy, France: 6 participants) have been involved in the workshop.

Results of this exchange showed important problems between readers (Mahé *et al.*, 2012). The aim of this workshop was to review the results of the exchange 2011 and compare them with those of the previous workshop, to review validation methods on these species, to clarify the interpretation of annual rings, to continue a reference collection of well defined otoliths and to improve the recommendations.

This report presented a review of age validation studies and state-of-the-art on ageing the two *Mullus* species. After the presentation of readings results (mean agreement percentage from 50.5% to 74.8%; mean CV from 29.7% to 61.7%) and the precision of age estimation, the participants identified the sources of bias in the interpretation of the *Mullus* age. In both species, the position of the first growth ring on the otolith was identified as most important problem especially for Mediterranean area. Regarding the age from scales and otoliths, the choice of calcified piece influenced the results. The participants agreed in indicating the otoliths as the best calcified structure to ageing *Mullus* species. After discussion, second reading was made with the new scheme. The precision increased specially for *M. barbatus* in the Mediterranean sea (mean agreement percentage from 58.7% to 76.5%; mean CV from 37.3% to 16.7%). Moreover, there were some advances on the guideline of age reading during this workshop but it could be necessary to continue to clarify this guideline especially in the Mediterranean sea for both species. In consequence, the participants of WKACM2 recommended study on validation methods for *Mullus* spp. ageing from otolith interpretation and also they indicated the proposals. Moreover, in 2014, new otoliths exchange will be organize. In 2015, the new workshop (WKCAM3; Palma de Mallorca; co-chairs : F. Ordines, Spain ; K. Mahé, France) will focus on the analysis of exchange results, validation studies and formalized guideline of ageing for *Mullus surmuletus* and *M. barbatus*.

1 Review on the biology

1.1 *Mullus barbatus* in the Mediterranean Sea

Red mullet, *Mullus barbatus*, is distributed in the eastern Atlantic, along the European and African coasts from the North Sea and England to Senegal and also in the Mediterranean and Black Seas (Fisher *et al.*, 1987). This species has a gregarious behaviour. In the Mediterranean Sea, red mullet is frequently found on muddy bottoms at depths ranging from 5 to 250 m. In summer, juveniles are concentrated very close to the shore; in autumn, they move towards deeper bottoms (Voliani, 1999). According to Voliani (1999), the maximum total length (TL) of red mullet in the Mediterranean Sea is 28–29 cm for females and 23 cm for males. Red mullet reproduction takes place near the coast, from May to June–July (Gharbi and Ktari, 1981; Cherif *et al.*, 2007). The length at first maturity is around 11–12 cm TL, both for females and males. Eggs, larvae and post larvae up to 30–35 mm of *M. barbatus* are pelagic and live in surface waters. According to Sabatés and Palomera (1987), larvae are found in strictly surface waters (0–1.5 m depth). Larvae are found in the Mediterranean Sea mainly between June and July (Sabatés and Palomera, 1987). Juveniles up to 4–5 cm TL are pelagic. Beyond this size, juveniles become demersal. Recruitment occurs in coastal bottoms in summer–early autumn period (Levi *et al.*, 2003). The maximum estimated age in the exploited standing stock is 10 years old for females and 7 for males.

1.2 *Mullus surmulutus* in the Northeast Atlantic and the Mediterranean Sea

The striped red mullet is found along the European coasts from South Norway (Wheeler, 1978) and North Scotland (Gordon, 1981) including Faeroes (Blacker, 1977), south to the northern part of western Africa and in the Mediterranean and Black Seas. It is infrequent off Norway, around Ireland, the north coasts of England and the West of Scotland (Pethon, 1979; Minchin and Molloy, 1980; Davis and Edward, 1988; Gibson and Robb, 1997).

In the Eastern Channel since 1988, young individuals are distributed in coastal areas, while the adults have a more offshore distribution in the east part (Mahé *et al.*, 2005). Finally, nurseries are located in Bay of Saint-Brieuc and at the Falklands coasts (Morizur *et al.*, 1996).

Striped red mullet is a benthic fish. It seems to prefer deep water and elevated temperatures, and tolerates weak and high salinity (corresponding respectively to the habitats of the juvenile and adults) and is rarely found in the transitions zones of intermediate salinity. This species prefer sandy sediments (Mahé *et al.*, 2005).

Growth study in the Eastern English Channel and the South of the North Sea (Mahé *et al.*, 2005) highlighted a sexual dimorphism expressing in a faster linear and ponderal growth for females than for males. Striped red mullet reproduce from May to September with an optimum in June in the North–East Atlantic (Mahé *et al.*, 2005) where it reached its size of first sexual maturity of 16.2 cm for males and 16.9 cm for females. This period of reproduction is the same as the one in Mediterranean Sea (Lalami, 1971 ; Hashem, 1973 ; Gharbi and Ktari, 1981).

2 Age Validation Studies (ToRb)

So far, any age validation studies for both species have been based on marking/tagging methods. Only indirect age validation methods have been used to observe date and formation of increment deposition.

2.1 *Mullus barbatus* in the Mediterranean Sea

Three studies have been focused on age validation by marginal increment analysis. The first one measured increment formation all year long and presents a lowest value of deposition in August and a highest in January, to conclude that the annual ring is formed after January and before August (EastMed, 2010 from Fisheries Laboratory, 1998).

The others studies were based on monthly observations of the marginal increment in order to investigate its formation, which leads to a deposition of a translucent zone from November to May and an opaque zone from June to September (Figure. 1 and 2). Marginal Increment Analysis has shown both a translucent and an opaque increments laid down each year.

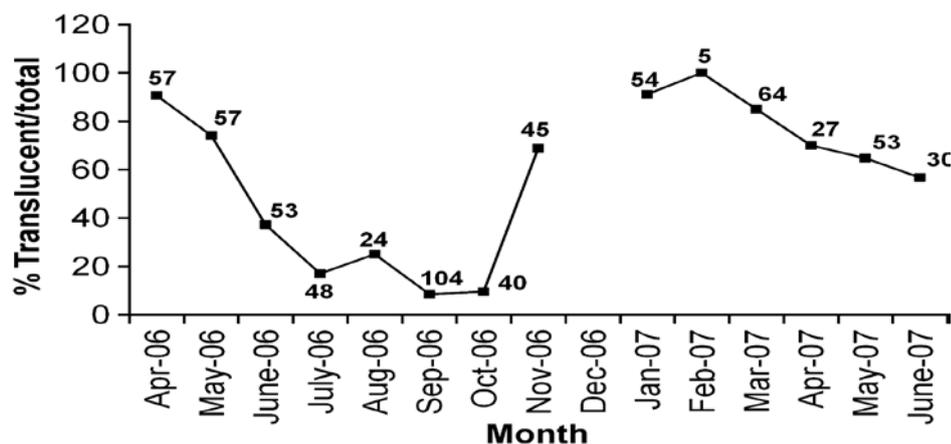


Figure 1: Percentage of occurrence (%) of translucent margins in *sagittae* of red mullet (*M. barbatus*). Number of specimens used to calculate the percentage by month (Sieli *et al.*, 2011).

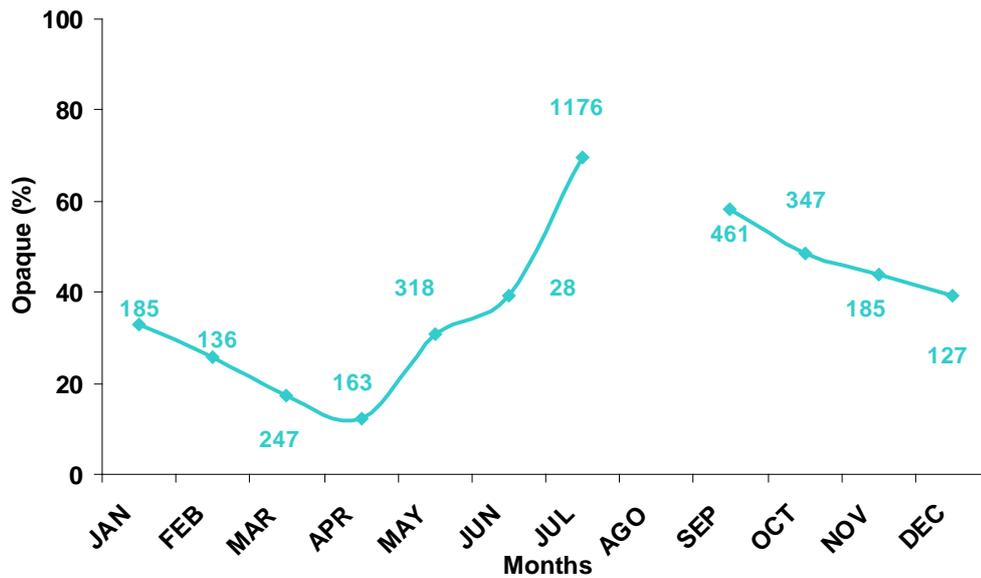


Figure 2: Percentage (%) of opaque margins in *sagittae* of red mullet (*M. barbatus*). Number of specimens used to calculate the percentage by month (tini, pers. comm.).

2.2 *Mullus surmuletus* in the Northeast Atlantic and Mediterranean Sea

Mahé *et al.*, (2005) analysed marginal increment to validate the periodicity of increment formation. Marginal-increment analysis (MI) was carried out on otoliths according to the following formula:

$$MI = (R - r_n) / (r_n - r_{n-1})$$

Where R is otolith ray, r_n is the ray of the last ring and r_{n-1} is the ray before the last ring.

It showed that months of lowest values run from February to April (Figure 3). Therefore, winter to spring appeared as the possible period of annulus formation. Similar results were found by Reñones *et al.* (1995) and Pajuelo *et al.* (1997) analysing the percentage of individuals with opaque edge (Figure 4 and 5).

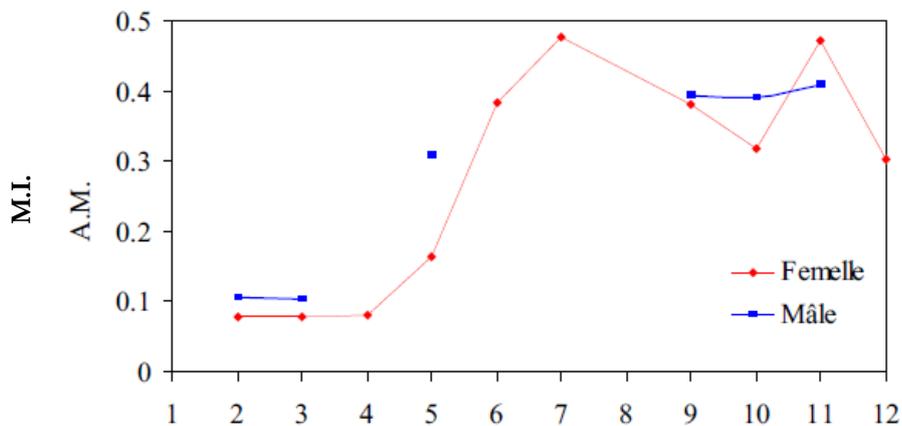


Figure 3: Marginal-increment (M.I.) per month of *M. surmuletus* in the Eastern English Channel and southern North sea (Mahé *et al.*, 2005).

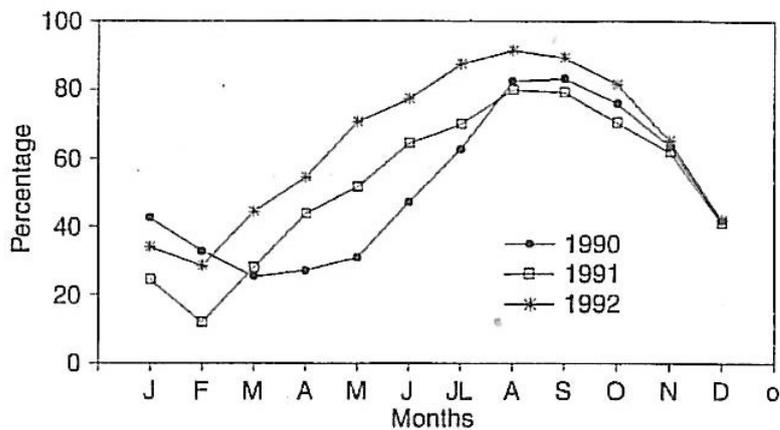


Figure 4: Monthly variations in percentage of individuals with opaque otolith marginal rings of *M. surmuletus* in 1990 (n=1220), 1991 (n=970), 1992 (n=1095) (Renones *et al.*, 1995).

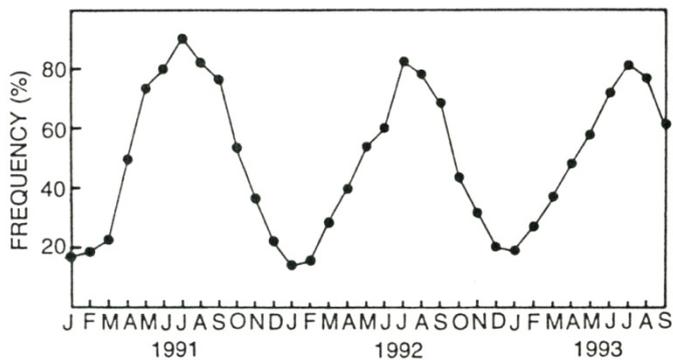


Figure 5: Monthly frequency of otoliths with opaque edges for *M. surmuletus* off the Canary Islands (Pajuelo *et al.*, 1997).

3 Results of reading (ToRa)

During these exchange and workshop, the samples used were not validated, therefore the « true age » is not known. In this way, the work group evaluates the precision of age estimation between readers but not the accuracy (Secor *et al.*, 1995; Panfili *et al.*, 2002 ; ICES, 2007) (Figure. 6).

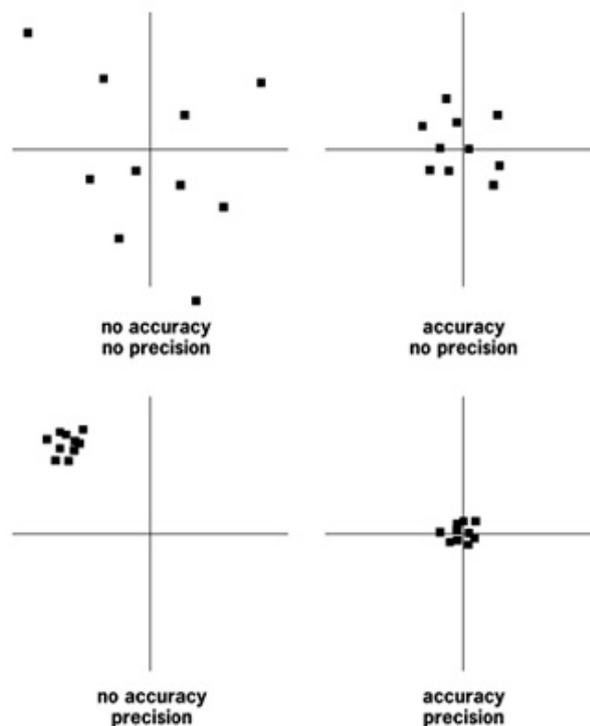


Figure 6: Accuracy¹ and precision² in the sclerochronological studies. The age estimation results (black boxes) are marked in relation to the true age value (tersection of axes X and Y). The accuracy corresponds to the proximity to the real value whereas the precision corresponds to the proximity of repeated measures (Panfili *et al.*, 2002).

The spreadsheet (Eltink, 2000) was used 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 defined as:

- Percentage agreement = $100 \times (\text{no. of readers agreeing with modal age} / \text{total no. of readers})$.
- Precision c. v. = $100 \times (\text{standard deviation of age readings} / \text{mean of age readings})$.

¹ In absence of calcified structures of known age, the age readings can be compared to modal age, which is defined as the age determined for an individual structure whose most of the readers have a preference. Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. The age reading comparisons to modal age provide a low estimate of relative bias compared to absolute bias, when most readers have a similar serious bias in age reading (ICES, 2007).

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).

3.1 First reading

During the exchange in 2011, the first reading was realized. Seven readers participated in this exchange (Table 1).

Table 1: List of the readers participated to the *M. surmuletus* and *M. barbatus* exchange.

| NAME | COUNTRY | INSTITUTE |
|-------------------------------------|---------|-----------|
| Romain Elleboode | France | IFREMER |
| Charis Charilaou | Cyprus | DFMR |
| Alessandro Ligas | Italy | CIBM |
| Carbonara Pierluigi / Intini Simona | Italy | COISPA |
| Francesc Ordinas / Natalia Gonzalez | Spain | IEO |

175 *Mullus surmuletus* and 202 *Mullus barbatus* were circulated to all Institutes :

Balearic Islands, *Mullus surmuletus*, whole and burned whole otoliths, 100 (IEO institute)

Balearic Islands, *Mullus surmuletus*, scales, 95 (IEO institute)

Bay of Biscay, *Mullus surmuletus*, whole and burned whole otoliths, 75 (IFREMER institute)

Southern Spain, *Mullus barbatus*, whole and burned whole otoliths, 100 (IEO institute)

Southern Spain, *Mullus barbatus*, scales, 68 (IEO institute)

Southern Adriatic sea, *Mullus barbatus*, whole otoliths, 102 (COISPA institute)

540 images with 377 otoliths and 163 scales were used (Figure 7 and 8; Table 2). 2 images sets for *Mullus surmuletus* in Balearic Islands and for *Mullus barbatus* in the Southern Spain have been realized to compare the age estimation between both calcified pieces.

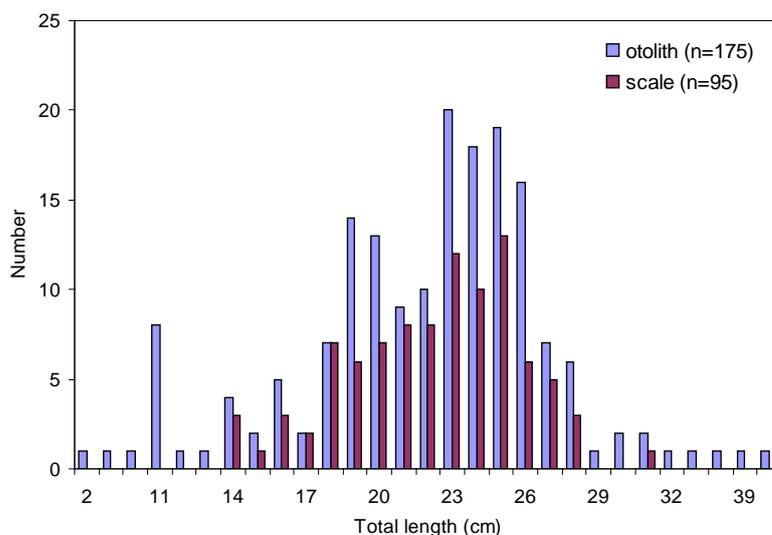


Figure 7: Histograms of the samples by calcified pieces of *Mullus surmuletus*.

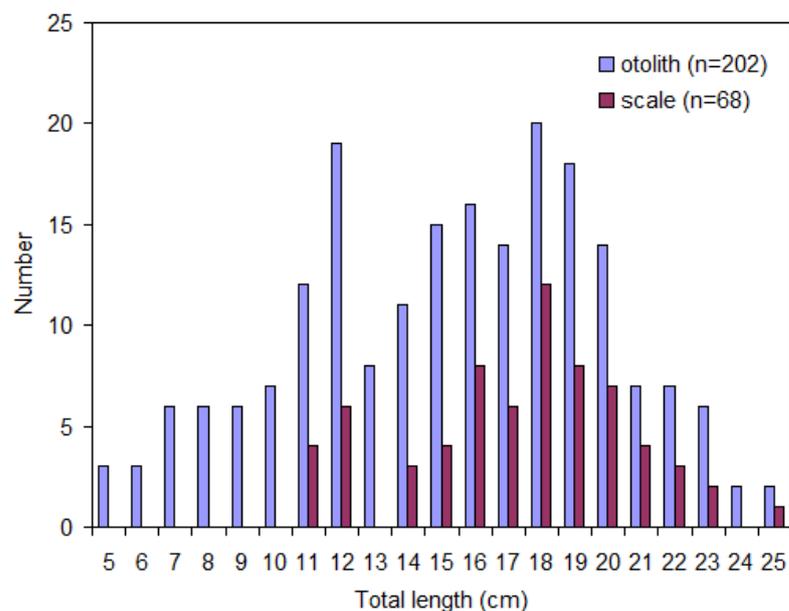


Figure 8: Histograms of the samples by calcified pieces of *Mullus barbatus*.

Table 2: Mean total length (range) of the fish (cm) by species, calcified piece and sex.

| SPECIES | CALCIFIED PIECE | FEMALE | IMMATURE | MALE | TOTAL |
|--------------------------|-----------------|---------------------|-----------------|---------------------|---------------------|
| <i>Mullus barbatus</i> | otolith | 17.6 (9–25.2) | 7.1 (5–8.5) | 14.6 (9–20.5) | 15.7 (5–25.2) |
| | scale | 18.3 (11.4–25.2) | | 15.5 (11.2–18.6) | 17.7 (11.2–25.2) |
| <i>Mullus surmuletus</i> | otolith | 23.2 (4–44) | 17.6 (11–28) | 22.3 (2–35) | 22.3 (2–44) |
| | scale | 23.9 (17.6–31.7) | | 19.5 (14–25.5) | 22.7 (14–31.7) |

The six samples were not read by all readers (Table 3).

Table 3: Number of readers by species and calcified piece.

| | <i>MULLUS SURMULETUS</i> | | <i>MULLUS BARBATUS</i> | |
|-------------------|--------------------------|-------|------------------------|-------|
| | whole otolith | scale | whole otolith | scale |
| Bay of Biscay | 6 | | | |
| Balearic Islands | 6 | 2 | | |
| Southern Spain | | | 6 | 2 |
| Southern Adriatic | | | 5 | |

Precision

Mean precision of age estimation for individual fish were Coefficient of Variation (CV, Table. 4) and percent agreement to modal age (Table. 5). Results among sets of calcified pieces showed large differences. The set of *Mullus surmuletus* otoliths from the Bay of Biscay presented the higher percentage agreement (74.8% ; Table 5). On 75 otoliths, 19 were read with 100% agreement (25%) and thus a CV of 0%. Modal age of these fish was comprised between 0 and 3 years. The second set with high percent agreement was composed by *Mullus barbatus* otoliths from the Southern Adriatic (71.9%; Table 5). On 102 otoliths, 34 were read with 100% percentage agreement (33%) and thus a CV of 0%.

The other sets of this exchange showed a very low precision, around 50% percentage agreement. The reason was not species because these results were for both species. In the Balearic Islands and the southern Spain, we compared readings from otoliths and scales but the difference between calcified pieces did not explain either these results. In Balearic Islands and the southern Spain, results from only otoliths presented respectively coefficients of 32.6% and 54.3% and percentage agreements of 54.1% and 54.2%.

Difference in precision could be largely due to the sampling area (it is easier to estimate age in Atlantic than in Mediterranean Sea) and also to the composition of the samples (most of the *Mullus barbatus* from Southern Adriatic presenting 100% percentage agreement at the age modal of 0 year. Inversely, modal age of fish from the Balearic Islands began to 1 year).

Table 4: Coefficient of Variation (range) for each set of images by species, calcified pieces and area.

| | <i>Mullus surmuletus</i> | | <i>Mullus barbatus</i> | |
|-------------------|--------------------------|-------|------------------------|-------|
| | whole otolith | scale | whole otolith | scale |
| Bay of Biscay | 61.7% (0-245%) | | | |
| Balearic Islands | 29.7% (12-65%) | | | |
| Southern Spain | | | 53.2% (11-155%) | |
| Southern Adriatic | | | 59.5% (0-173%) | |

Table 5: Percent agreement (range) for each set of images by species, calcified pieces and area.

| | <i>Mullus surmuletus</i> | | <i>Mullus barbatus</i> | |
|-------------------|--------------------------|-------|------------------------|-------|
| | whole otolith | scale | whole otolith | scale |
| Bay of Biscay | 74.8% (33-100%) | | | |
| Balearic Islands | 52.2% (20-86%) | | | |
| Southern Spain | | | 50.5% (17-88%) | |
| Southern Adriatic | | | 71.9% (0-100%) | |

Relative bias (Accuracy)

The minimal requirement for age reading's consistency is 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. This table shows inter-reader bias test and reader against modal age bias test (–: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

The tables 6, 7, 8 and 9 showed these analyses for the set coming from different areas and from both species of *Mullus*.

Table 6: Inter-reader bias test and reader against modal age bias test for the set of *Mullus surmuletus* coming from the Bay of Biscay.

| | France RE Reader 1 | Italy AL Reader 2 | Italy CI Reader 3 | Spain FO Reader 4 | Spain NG Reader 5 | Cyprus CC Reader 6 |
|------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| Reader 1 | | | | | | |
| Reader 2 | * | | | | | |
| Reader 3 | – | * | | | | |
| Reader 4 | ** | ** | ** | | | |
| Reader 5 | ** | ** | ** | – | | |
| Reader 6 | * | ** | ** | ** | ** | |
| MODAL age | – | * | – | ** | ** | ** |

Table 7: Inter-reader bias test and reader against modal age bias test for the set of *Mullus surmuletus* coming from the Balearic Islands.

| | France RE Reader 1 | Italy AL Reader 2 | Italy CI Reader 3 | Cyprus CC Reader 4 | Spain FO Reader 5 | Spain NG Reader 6 | France RE Reader 7 | Italy AL Reader 8 |
|------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|
| Reader 1 | | | | | | | | |
| Reader 2 | ** | | | | | | | |
| Reader 3 | ** | ** | | | | | | |
| Reader 4 | ** | ** | ** | | | | | |
| Reader 5 | ** | – | ** | ** | | | | |
| Reader 6 | ** | ** | – | ** | ** | | | |
| Reader 7 | ** | ** | ** | ** | ** | ** | | |
| Reader 8 | ** | ** | – | ** | ** | – | ** | |
| MODAL age | ** | ** | – | ** | ** | ** | ** | – |

Table 8: Inter-reader bias test and reader against modal age bias test for the set of *Mullus barbatus* coming from the southern Spain.

| | France RE Reader 1 | Italy AL Reader 2 | Italy CI Reader 3 | Cyprus CC Reader 4 | Spain FO Reader 5 | Spain NG Reader 6 | France RE Reader 7 | Italy AL Reader 8 |
|------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|
| Reader 1 | | | | | | | | |
| Reader 2 | * * | | | | | | | |
| Reader 3 | * * | * * | | | | | | |
| Reader 4 | * * | * * | * * | | | | | |
| Reader 5 | * * | — | * * | * * | | | | |
| Reader 6 | * * | — | * * | * * | — | | | |
| Reader 7 | * * | * * | * * | * * | * * | * * | | |
| Reader 8 | * * | * | * * | * * | — | — | * * | |
| MODAL age | * * | * * | * * | * * | * * | * * | * * | — |

Table 9: Inter-reader bias test and reader against modal age bias test for the set of *Mullus barbatus* coming from the southern Adriatic sea.

| | France RE Reader 1 | Italy AL Reader 2 | Spain FO Reader 3 | Spain NG Reader 4 | Italy CI Reader 5 |
|------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|
| Reader 1 | | | | | |
| Reader 2 | * * | | | | |
| Reader 3 | * * | * | | | |
| Reader 4 | * * | * * | — | | |
| Reader 5 | * * | * * | * * | * * | |
| MODAL age | * * | — | * * | * * | * * |

It should be noted that there were certainty of bias among readings from otoliths and from scales and modal age. Moreover, there is certainty of bias between the readings from different calcified pieces of the same fish.

3.2 Age reading quality

Age reading quality was estimated by all readers (Table 10).

Table 10: Level of Age reading quality by readers and all readers of the otoliths (readers 1, 2, 3, 4) and scales (reader 6) by species and by areas.

| SPECIES | AREA | LEVEL OF QUALITY | READER 1 | READER 2 | READER 3 | READER 4 | READER 6 | TOTAL | |
|----------------------|-------------------|------------------|----------|----------|----------|----------|----------|-------------|-------------|
| <i>M. surmuletus</i> | Bay of Biscay | AQ1 | | 3 | 20 | | | 23 (7.6%) | |
| | | AQ2 | 75 | 70 | 55 | 75 | | 275 (91.6%) | |
| | | AQ3 | | 2 | | | | 2 (0.6%) | |
| | Balearic Islands | AQ1 | | | | 11 | | 5 | 16 (4.1%) |
| | | AQ2 | | 36 | 78 | 100 | 40 | | 254 (64.8%) |
| | | AQ3 | | 64 | 8 | | 50 | | 122 (31.1%) |
| <i>M. barbatus</i> | Southern Spain | AQ1 | | 1 | 29 | | | 31 (15.0%) | |
| | | AQ2 | | 78 | 54 | | | 134 (65.0%) | |
| | | AQ3 | | 21 | 17 | | | 41 (19.9%) | |
| | Southern Adriatic | AQ1 | | 30 | 6 | | | | 36 (12.1%) |
| | | AQ2 | | 52 | 64 | | | | 116 (39.0%) |
| | | AQ3 | 99 | 20 | 26 | | | | 145 (48.8%) |

Images of *Mullus surmuletus* in the Bay of Biscay were almost all classified into AQ2 (91.6%). For the others sets, Quality level 2 decreased for Quality level 3 (difficult to age with acceptable precision).

3.3 Second reading

During the workshop, the second reading was realized. 4 readers participated in this exchange (Italy: 2; France : 1; Spain : 1). 50 images per species (*Mullus surmuletus* from Balearic Islands ; *Mullus barbatus* from Southern Spain) were analysed with TNPC software (www.tnpc.fr).

Results between the first and the second readings (with the same set and the same readers) showed that precision increase but difference between both readings were more important for *M. barbatus* than for *M. surmuletus* (Table 11).

Table 11: Percentage of agreement and Coefficient of Variation (mean with range) of the first and the second reading by species and by area.

| SPECIES | AREA | VALUE | FIRST READING | SECOND READING |
|----------------------|-----------------|-------------|---------------|----------------|
| <i>M. surmuletus</i> | Balearic island | % Agreement | 58.3 (25–100) | 65 (0–100) |
| | | CV | 23 (0–56) | 17.4 (0–37) |
| <i>M. barbatus</i> | Southern Spain | % Agreement | 58.7 (33–100) | 76.5 (50–100) |
| | | CV | 37.3 (0–115) | 16.7 (0–77) |

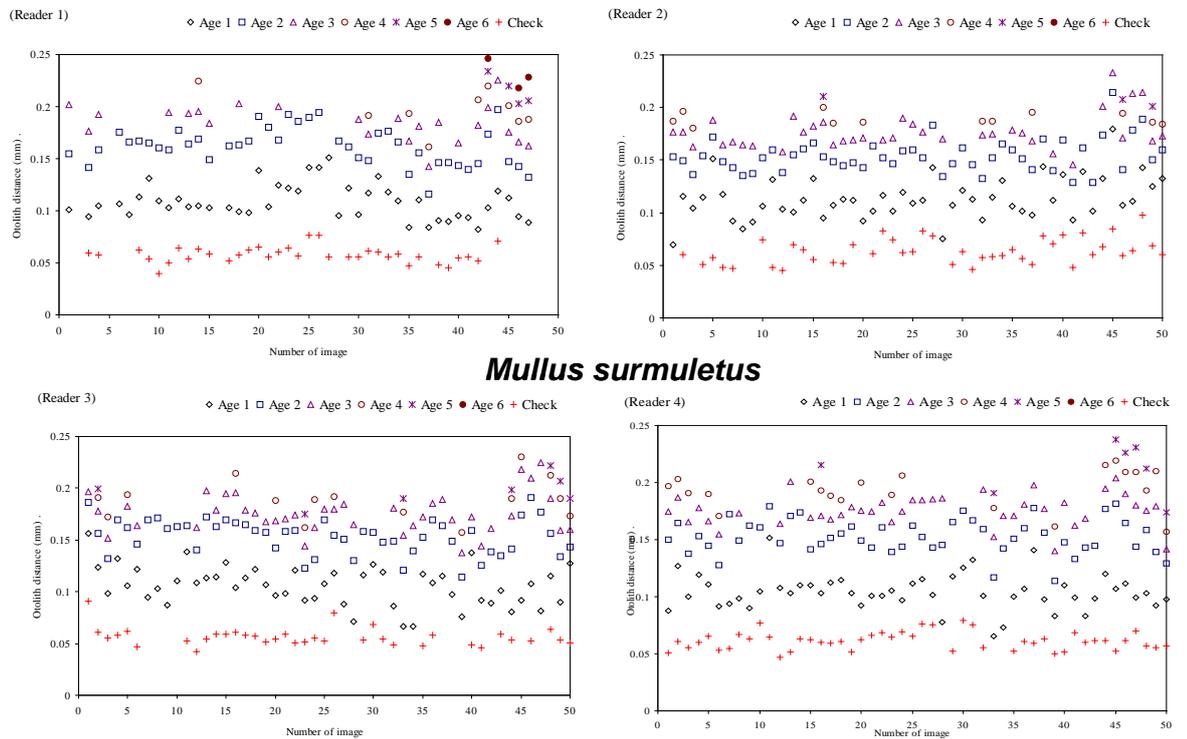
The tables 12 and 13 showed these analyses for the set coming from different areas and from both species of *Mullus*. To compare modal age to age of each reader, there is only one reader with possibility of bias ($0.01 < p < 0.05$) for *M. surmuletus* and no bias for *M. barbatus*.

Table 12: Inter-reader bias test and reader against modal age bias test for the set of *Mullus surmuletus* coming from the Balearic Islands.

| | esp Reader 1 | fr Reader 2 | it AL Reader 3 | it PL Reader 4 |
|------------------|-----------------|----------------|-------------------|-------------------|
| Reader 1 | | | | |
| Reader 2 | — | | | |
| Reader 3 | — | ** | | |
| Reader 4 | * | ** | — | |
| MODAL age | — | — | — | * |

Table 13: Inter-reader bias test and reader against modal age bias test for the set of *Mullus barbatus* coming from the southern Spain.

| | esp Reader 1 | fr Reader 2 | it AL Reader 3 | it PL Reader 4 |
|-----------|-----------------|----------------|-------------------|-------------------|
| Reader 1 | | | | |
| Reader 2 | — | | | |
| Reader 3 | * | * * | | |
| Reader 4 | * | * * | — | |
| MODAL age | — | — | — | — |



During the second reading, all readers used TNPC software in order to measure all distances between *nucleus* to the edge on this axis from *nucleus* to the posterior area of the otolith (see Guideline for age interpretation). Distances analysis will provide tools to identify mismatches between readings and/or readers (Figure 9).

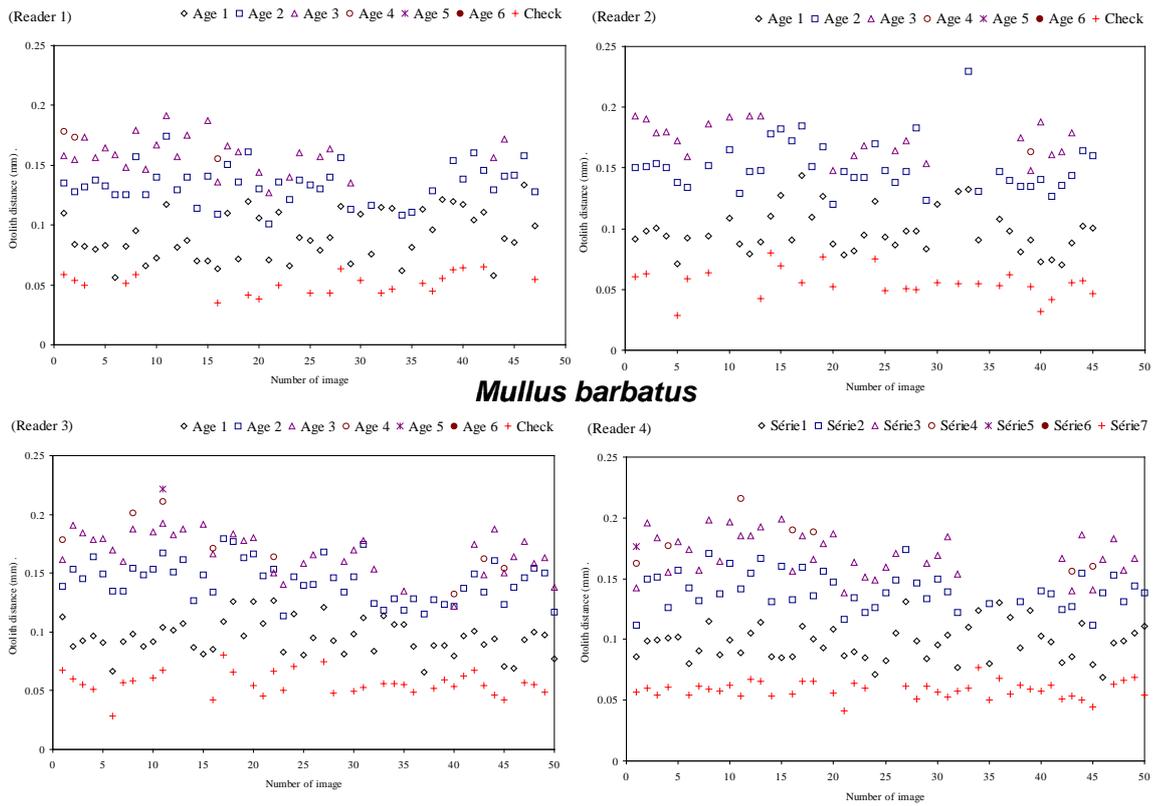
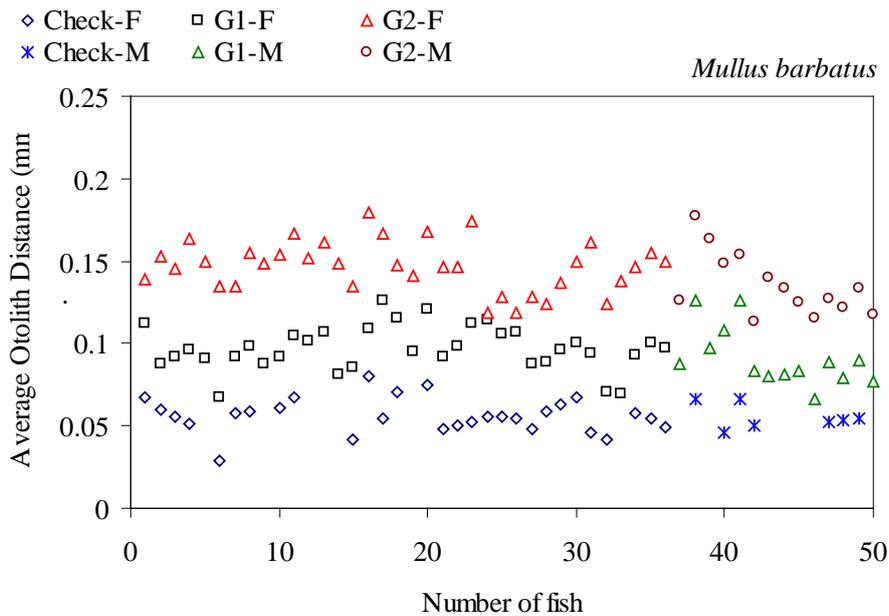


Figure 9: Otolith distance (mm) for each ring by species and by reader.

All distances, between *nucleus* to rings (false or growth rings), were well identified for both species, especially for the three first rings (check and the two first rings as annual growth) (Figure 10). Otolith of *M. surmuletus* don't show overlap on the three first rings, contrarily to *M. barbatus* where overlaps appeared on the check with the first ring as well as the first to the second one. Average otolith distance didn't showed difference between male and female but the male numbers in the samples were small.



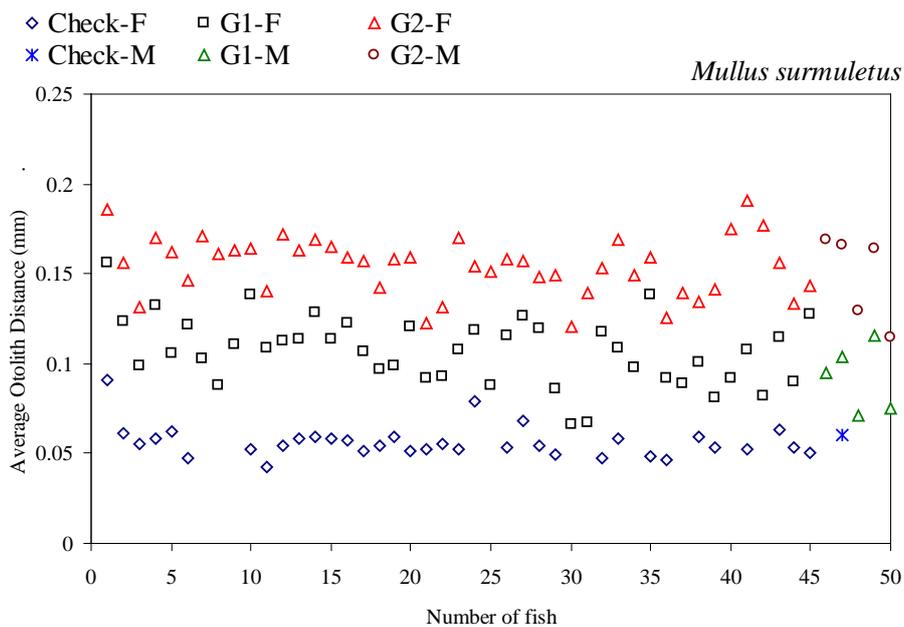


Figure 10: Average otolith distance (mm) for each ring by species and by sex (F : Female; M : Male).

Image analysis shows that distances between rings are similar from old either juveniles individuals otoliths (Figure 11).

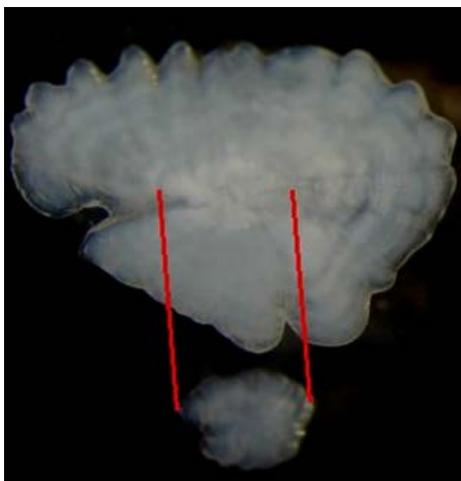


Figure 11: Otoliths of *Mullus barbatus* from Central Southern Tyrrhenian sea, Up : total length 28 cm, month capture May, Down : total length 4 cm, month capture August.

4 Sources of bias (ToRb)

4.1 *Mullus surmuletus*

During the workshop, the following remarks were pointed out from the discussion:

- Disagreement in the identification of the first annual ring; one group of the readers considered the first ring as a ring of settlement, whereas the majority considered it as the first annual ring.
- Disagreement in the identification of other rings.
- Confusion concerning the reading protocol during the exchange; some of the readers considered as date of birth the 1st of January, whereas others considered the 1st of July as date of birth.

4.2 *Mullus barbatus*

Discussion during the workshop revealed following remarks:

- Disagreement in the identification of the first annual ring; there was a high variability of what was considered as first annual ring. As a result, the radius of the first annulus presented a great variability between readers. Some of the readers considered the first very clear ring as a ring of settlement.
- Confusion concerning the reading protocol during the exchange; some of the readers considered as date of birth the 1st of January, whereas others considered the 1st of June as date of birth.

4.3 Position of the first growth ring

Sieli *et al.* (2011) considered the first two translucent marks, which can also appear as double marks separated by a large translucent zone, to be a pelagic and demersal checks and were not considered in the age estimation (Figure 12).

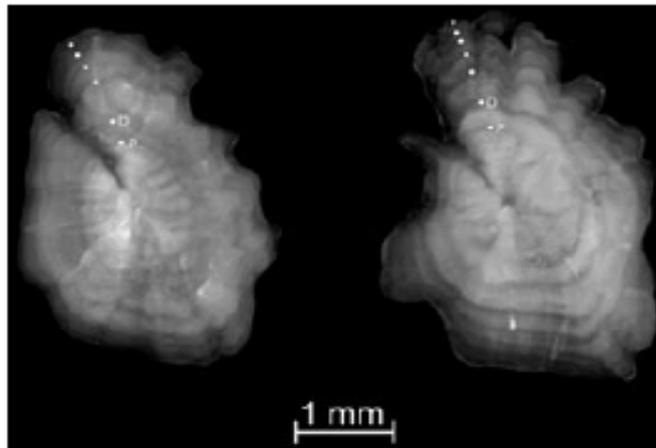


Figure 12: Whole sagitta reading of 4-year (on left) and 5-year (on right) red mullet (*M. barbatus*) females (200 and 220 mm TL, respectively). Translucent rings having a yearly meaning marked with white circle. The pelagic (P) and demersal (D) checks are also reported (Seili *et al.*, 2011).

During the first workshop on the age of *M. surmuletus* and *M. barbatus*, there was confusion between readers about the position of the first growth ring and the false ring (Figure 13).

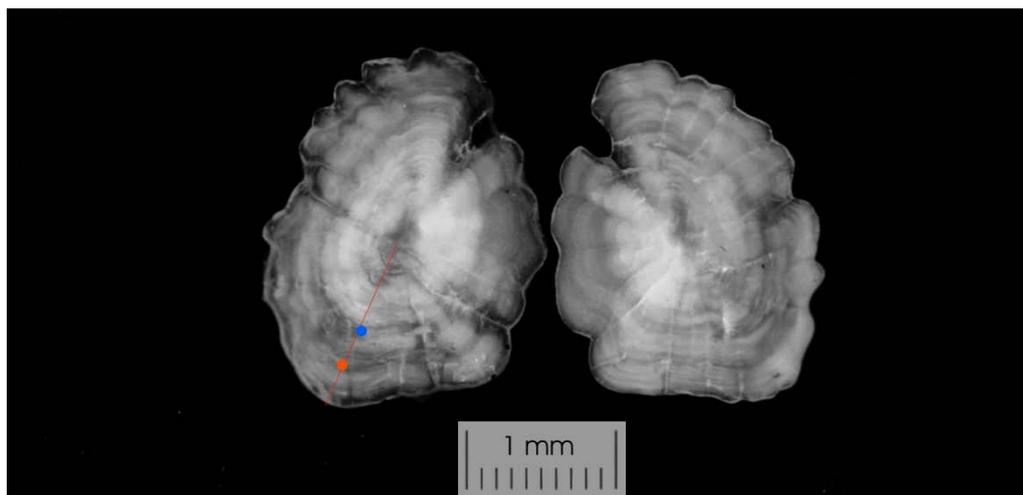


Figure 13: Image of *M. barbatus* (blue : the first growth ring or a false ring ; In orange : the growth ring for all readers; (ICES, 2009).

This first ring considered as “demersal” check is an important problem because we have no information to consider this ring as false or growth ring. Validation study as age estimation on juveniles by daily increments analysis could help to identify the first annual growth ring.

4.4 Choice of the Calcified Piece

A lot of studies were realized on age of *M. surmuletus* (Table 14) and *barbatus* (Table 15). At first, studies used scales but since the 80's, age is estimated by otoliths. During this exchange, the estimated age by the same reader showed certainty of bias ($p < 0.01$) between otoliths and scales. This difference was explained by the difficulty to interpret the edge of the scales for big *Mullus*, according to Mahé *et al.*, 2005.

Table 14: Chronological list of growth studies of *Mullus surmuletus* by area and by used calcified piece (from Mahé *et al.*, 2005).

| AREA STUDY | OTOLITHS | SCALES | AUTHORS | YEAR OF PUBLICATION |
|-----------------------------------------|----------|--------|-----------------------|---------------------|
| Bay of Biscay | | * | Desbrosses | 1935 |
| Algerian mediterranean waters | | * | Bougis | 1952 |
| Egyptian mediterranean waters | | * | Hashem | 1973 |
| Tunisia waters | | * | Gharbi and Ktari | 1981 |
| Catalonia waters | | * | Sanchez <i>et al.</i> | 1983 |
| Mallorca waters | * | | Morales–Nin | 1992 |
| Mallorca waters | * | | Renõnes <i>et al.</i> | 1995 |
| Canary Islands | * | | Pajeulo <i>et al.</i> | 1997 |
| Tunisia waters (Gabès bay) | * | | Jabeur <i>et al.</i> | 2000 |
| Eastern Channel– South of the North Sea | * | * | Mahé <i>et al.</i> | 2005 |
| Bay of Biscay | * | | N'Da <i>et al.</i> | 2006 |
| Egyptian mediterranean waters | * | | Mehanna | 2009 |

Table 15: Chronological list of growth studies of *Mullus barbatus* by area and by used calcified piece (from Bianchini and Ragonese, 2011).

| AREA STUDY | OTOLITHS | SCALES | AUTHORS | YEAR OF PUBLICATION |
|-------------------------------|----------|--------|-----------------------------------|---------------------|
| Bay of Biscay | | * | Desbrosses | 1935 |
| Adriatic sea | | * | Scaccini | 1947 |
| Algerian mediterranean waters | | * | Bougis | 1952 |
| Israel | * | | Wirszurbski | 1953 |
| Israel | | | Gotlieb | 1956 |
| Spanish coast | | * | Planas and Vives | 1956 |
| Spanish coasts (Catalan sea) | | * | Suau and Vives | 1957 |
| Gulf of Lions | * | * | Passalaigne | 1974 |
| Athens Gulf | * | | Papacostantinou <i>et al.</i> | 1981 |
| Tunisia waters | | * | Gharbi and Ktari | 1981 |
| Cyprus | * | | Livadas | 1985 |
| Strait of Sicily | * | | Andaloro and Prestipino Giaritta | 1985 |
| Cyprus | * | * | Livadas | 1989 |
| Aegean sea | * | | Vassilopoulou and Papacostantinou | 1992 |
| North coasts of Sicily | * | | Potoschi <i>et al.</i> | 1993 |
| Ionian sea | * | | Tursi <i>et al.</i> | 1996 |
| Ligurian Sea | * | | Fiorentino <i>et al.</i> | 1998 |
| Sardinia | * | | Sabatini <i>et al.</i> | 2002 |
| Strait of Sicily | * | | Sonin <i>et al.</i> | 2007 |

5 Guidelines for age interpretation (ToRc)

- 1) Recommended calcified piece to age *Mullus* is otolith.
- 2) As a first step, a blind reading shall be performed at the beginning without any information except the date of capture.
- 3) Selection of a suitable measurement axis; it is proposed the axis joining the *sulcus* and the *nucleus* of the otolith (Figure 14). Measurements shall be performed on this axis from *nucleus* to the posterior area of the otolith. This axis must be constant for all measurements.

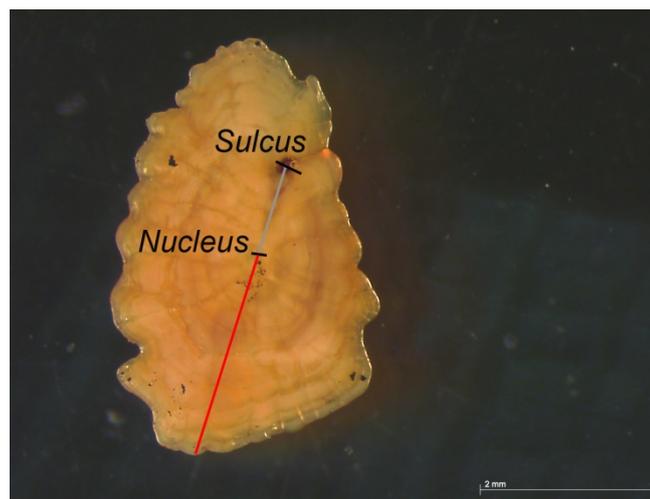


Figure 14: The suitable measurement axis from the *sulcus* to the *nucleus* and measure from *nucleus* to the posterior area of otolith (red line).

- 4) Annotate all considered false or true rings and measure them, if possible without any idea of these measurements (it depends of the used software).
- 5) Translucent true rings should be visible more or less around the whole otolith in order to be considered as annual rings.
- 6) In the Atlantic, 1st of January is considered as the date of birth. As a result, if a translucent ring is observed at the edge of the otolith at the first part of the year, then it shall be counted as annulus. In contrary, if a translucent ring is observed at the edge of the otolith at the second part of the year, then it should not be counted as *annulus* (Figure 15).

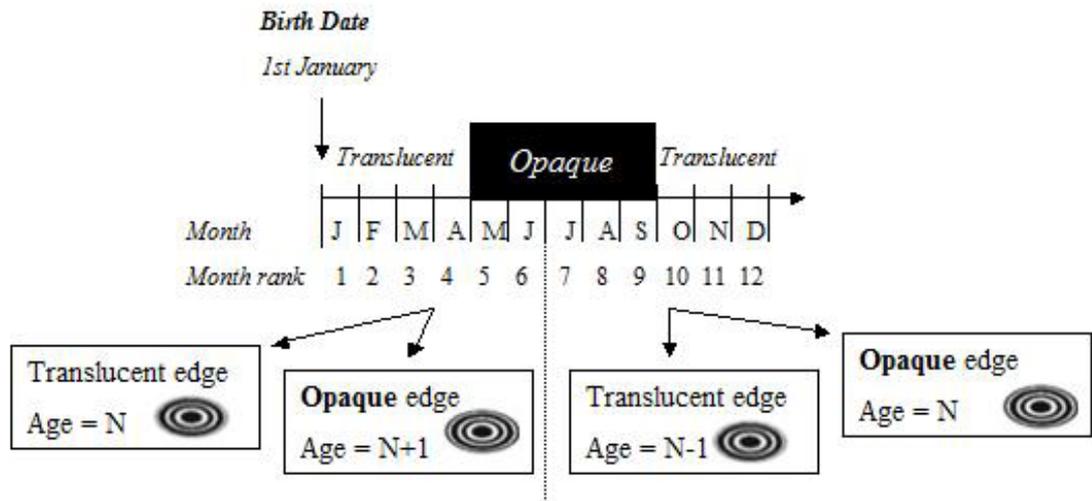


Figure 15: Approach of *Mullus* age (years; From Morales–Nin and Panfili, 2002 ; Mahé *et al.*, 2009) from otoliths reading in Atlantic. N is a number of translucent areas. Conventionally, the birth date is fixed at the 1st January as the birth date for all individuals (Williams and Bedford, 1974).

- 7) In the Mediterranean Sea, age interpretation is realized as Atlantic or with another approach (Figure 16). In this area, 2 approaches are in contradiction concerning the conventional birth date and assignment of age estimation.

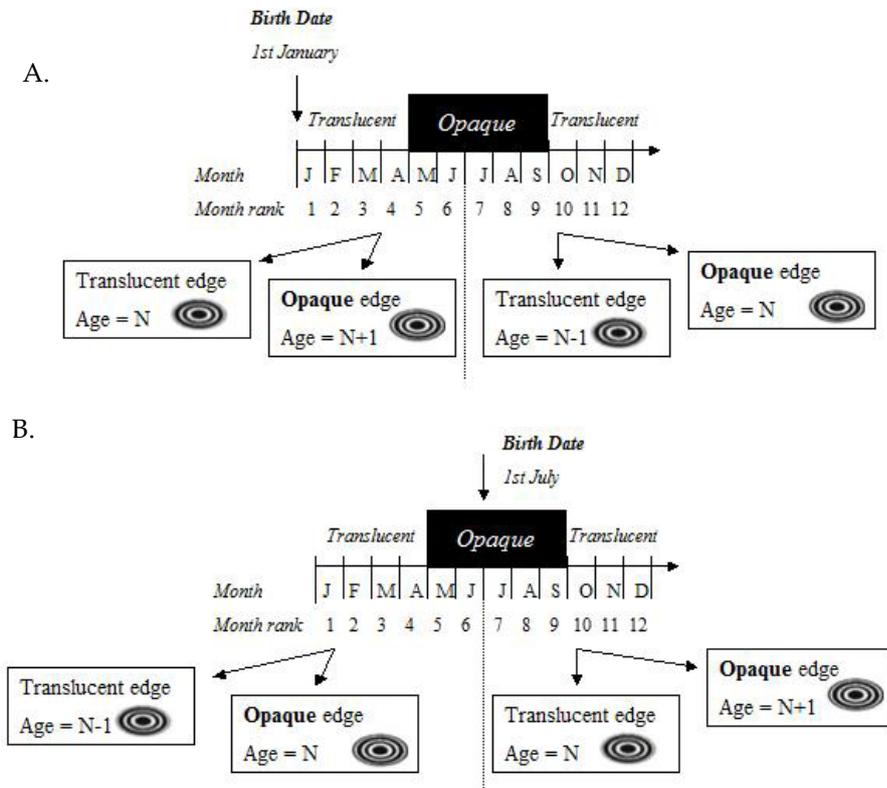


Figure 16: Approaches of *Mullus* Age (years) (From Morales–Nin and Panfili, 2002 ; Mahé *et al.*, 2009) from otoliths reading in Mediterranean Sea. N is a number of translucent areas.

- 8) Evaluate results of the first blind reading, examine the variability of presence of *annuli* and its measurements.
- 9) Use various validation methods such as back-calculation, marginal increment analysis, examination of the growth increment between the consecutive rings.
- 10) Evaluate all these results while taking into consideration available biological informations concerning the species such as, period of presence of new recruits and their lengths, modes in length composition of the stock, period of annulus formation.

6 Reference collection of otoliths (ToRd)

As agreement in age reading was low among readers for both *Mullus* species, few images were selected as good examples to start create a reference collection. The following images were chosen by all readers:

6.1 *M. surmuletus*



Figure 19 : Image of *M.surmuletus* (age : 1 ; length : 280 mm , Sex : M , month of capture : 10) from the bay of Biscay.



Figure 20 : Image of *M.surmuletus* (age : 3 ; length : 340 mm , Sex : F , month of capture : 10) from the bay of Biscay.



Figure 21 : Image of *M.surmuletus* (age : 2 ; length : 350 mm , Sex : F , month of capture : 10) from the bay of Biscay.



Figure 22 : Image of *M.surmuletus* (age : 4 ; length : 360 mm , Sex : F , month of capture : 10) from the bay of Biscay.

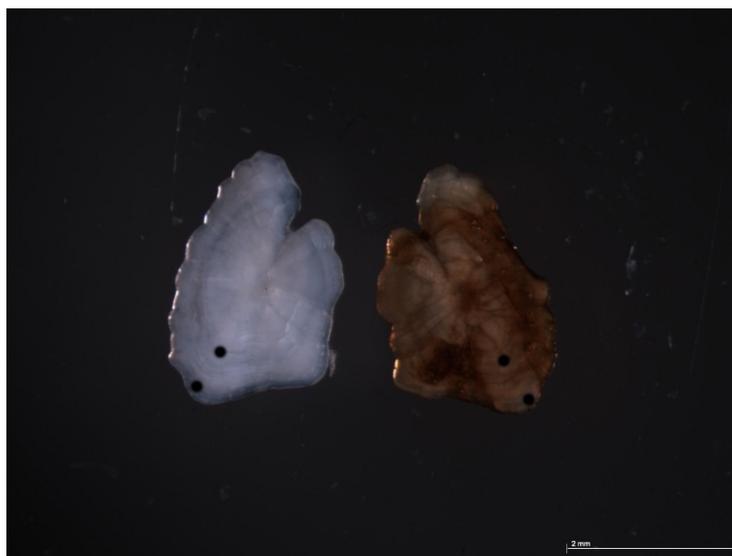


Figure 22: Image of *M. surmuletus* (age: 2; length: 161 mm, Sex: M, month of capture: 3) from southern Spain.

6.2 *M. barbatus*

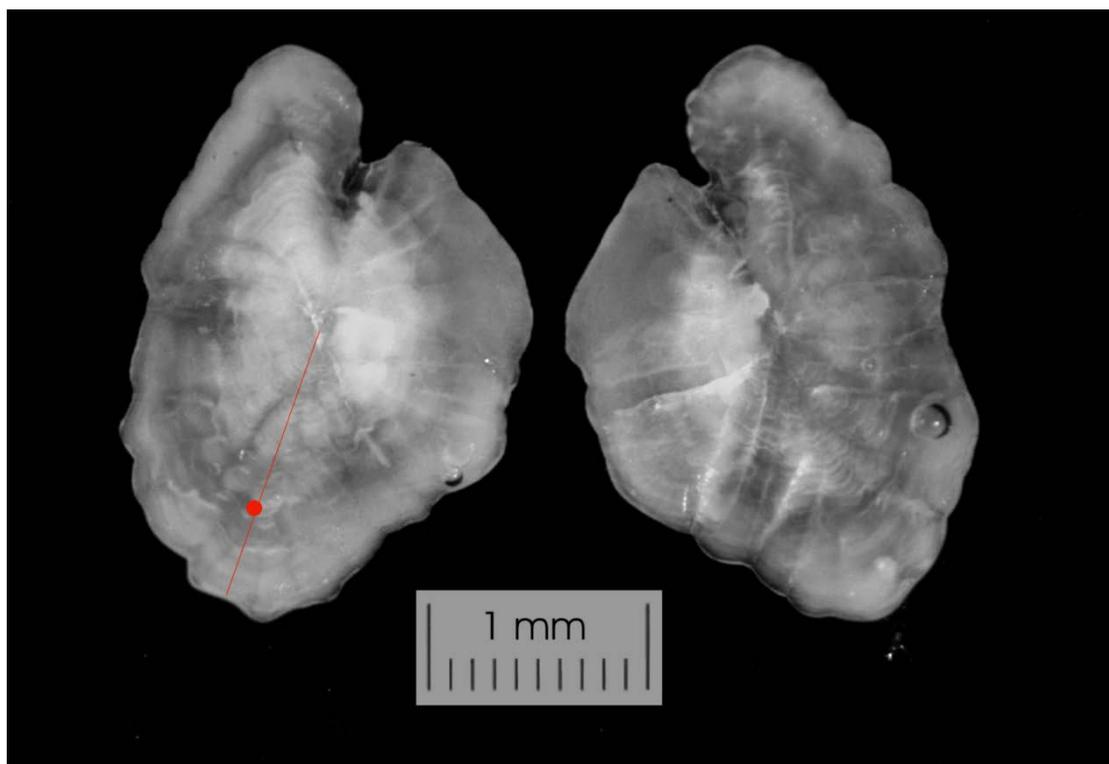


Figure 23: Image of *M. barbatus* (age: 1; length: 130 mm, Sex: M, month of capture: 7) from Strait of Sicily.

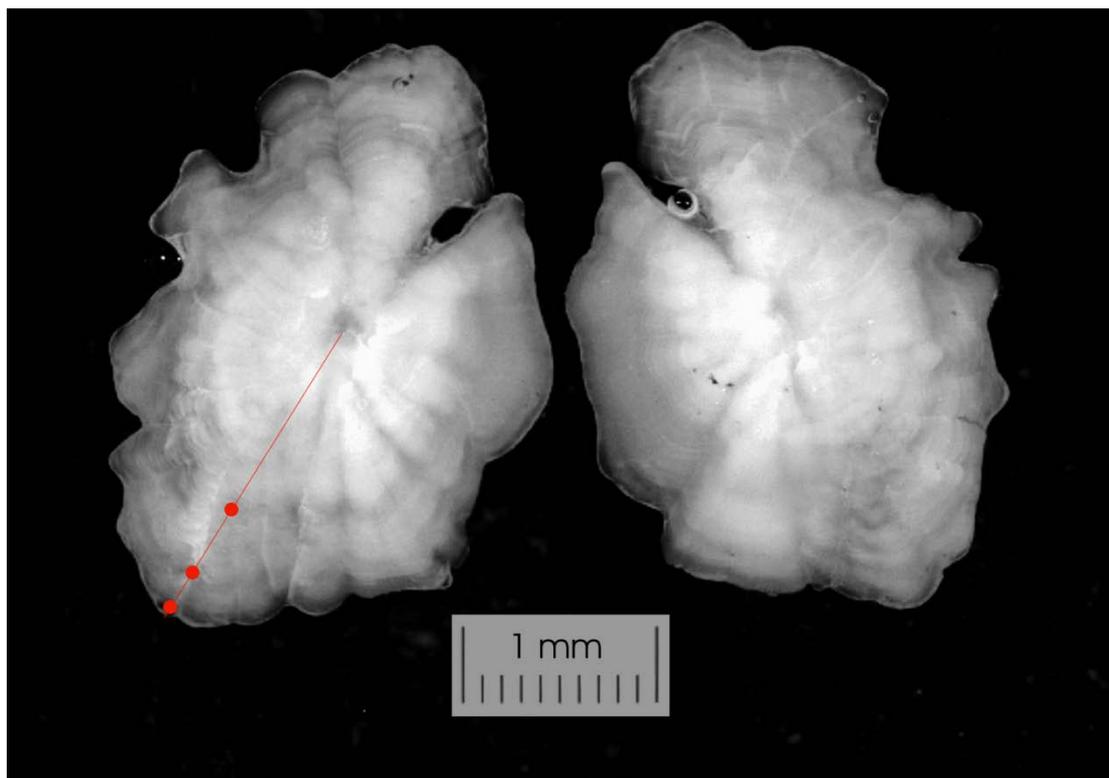


Figure 24: Image of *M. barbatus* (age: 3; length: 160 mm, Sex: M, month of capture: 6) from Strait of Sicily.

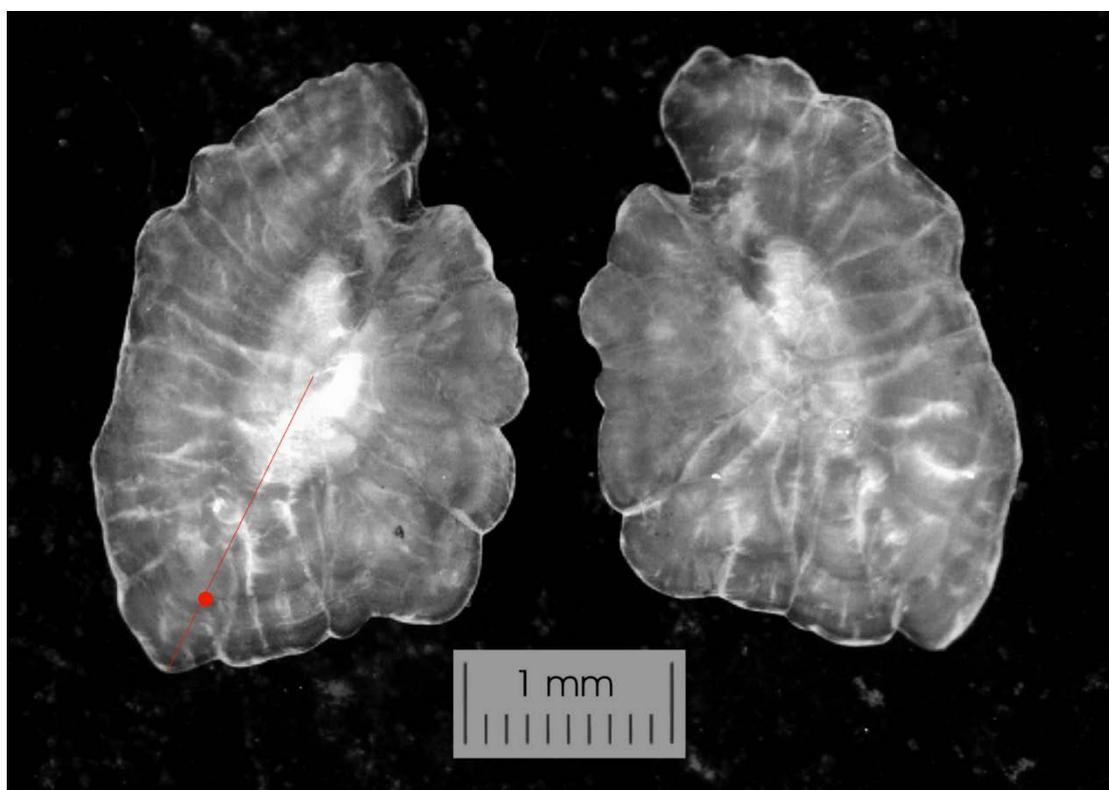


Figure 25: Image of *M. barbatus* (age: 1; length: 155 mm, Sex: M, month of capture: 11) from Strait of Sicily.

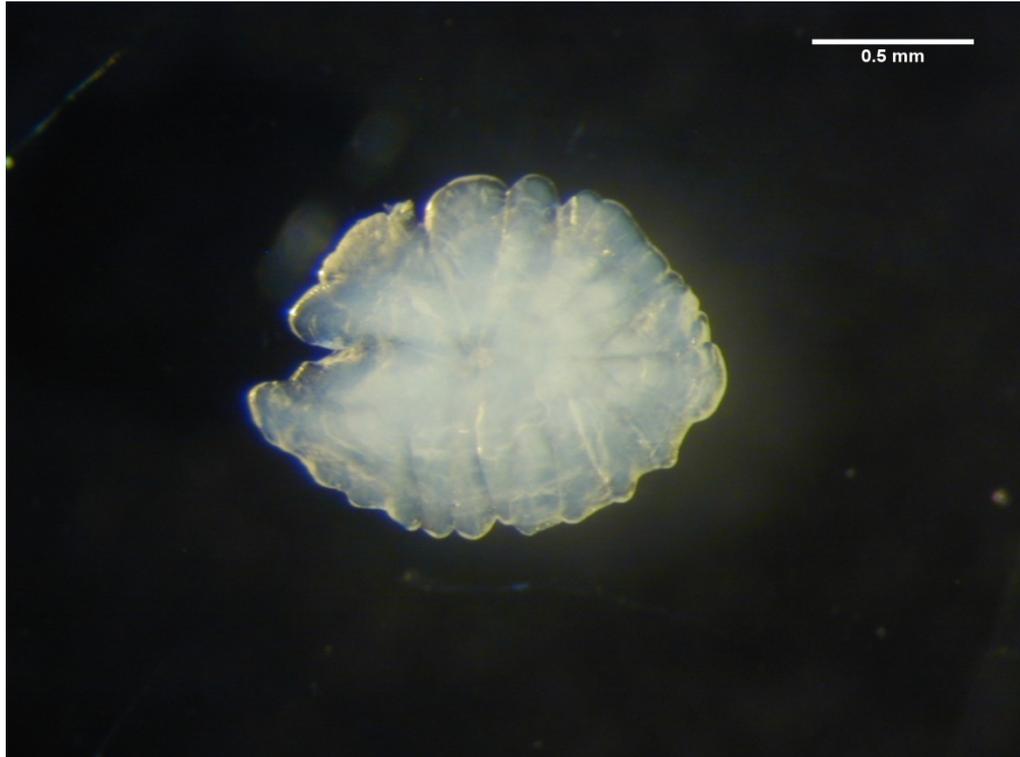


Figure 26: Image of *M. barbatus* (age: 0; length: 55 mm, Sex: I, month of capture: 9) from south of Adriatic.

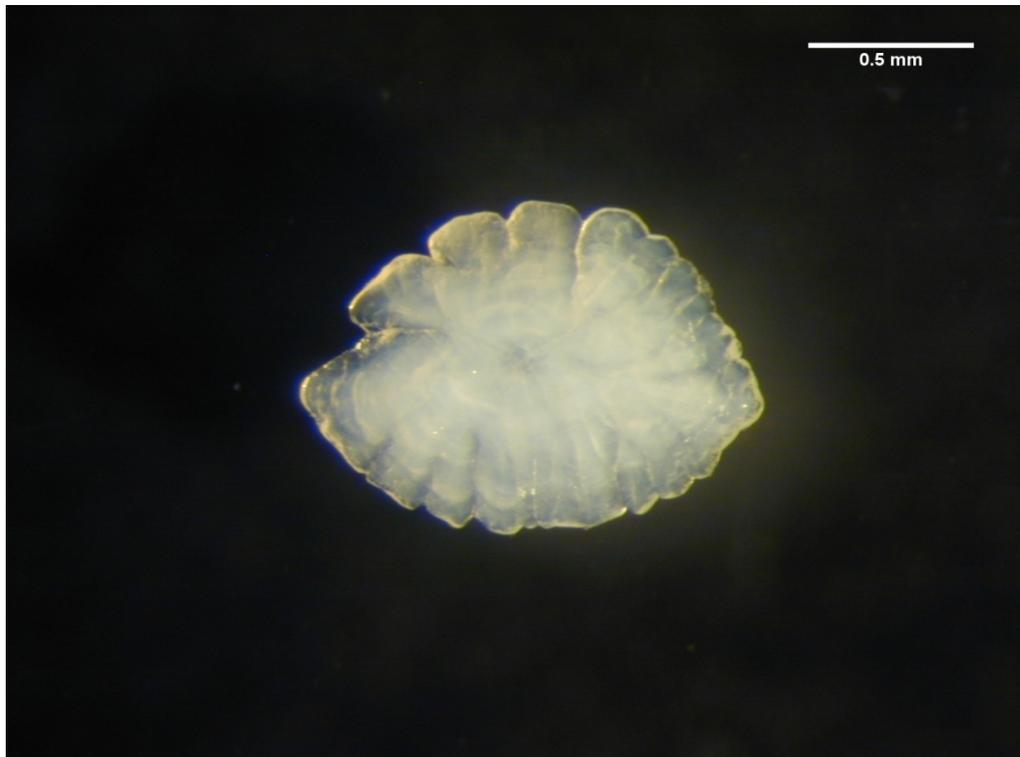


Figure 27: Image of *M. barbatus* (age: 0; length: 50 mm, Sex: I, month of capture: 9) from south of Adriatic.



Figure 28: Image of *M. barbatus* (age: 3; length: 215 mm, Sex: F, month of capture: 3) from south of Adriatic.

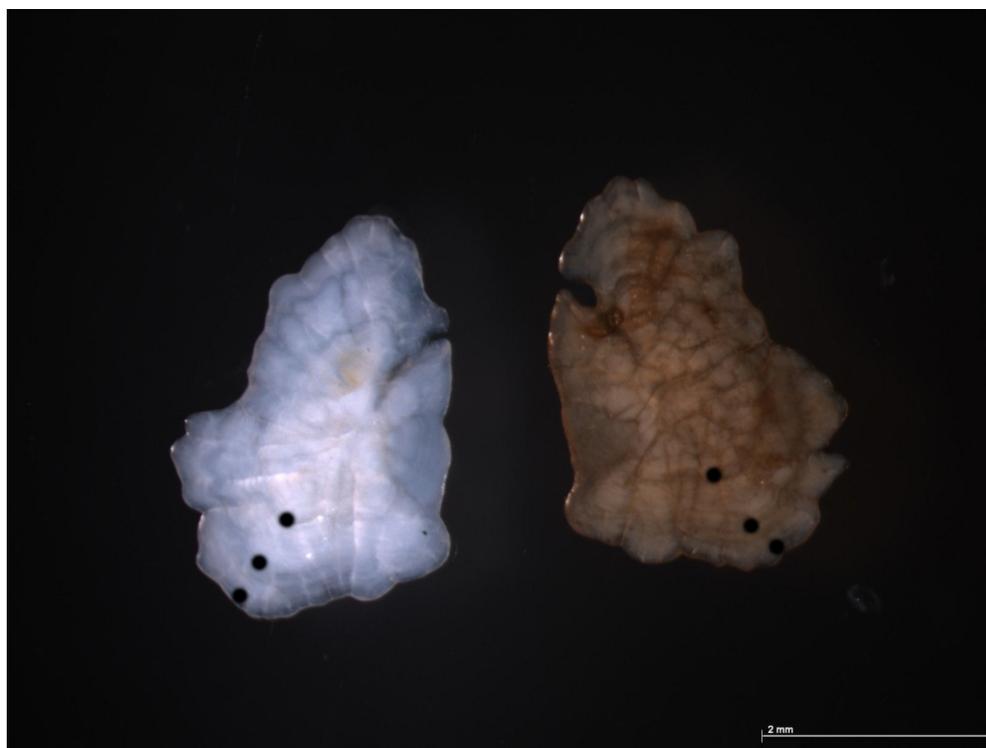


Figure 29: Image of *M. barbatus* (age: 3; length: 210 mm, Sex: F, month of capture: 2) from Balearic Islands.



Figure 30: Image of *M. barbatus* (age : 2 ; length : 183 mm , Sex : F , month of capture : 2) from Balearic islands.

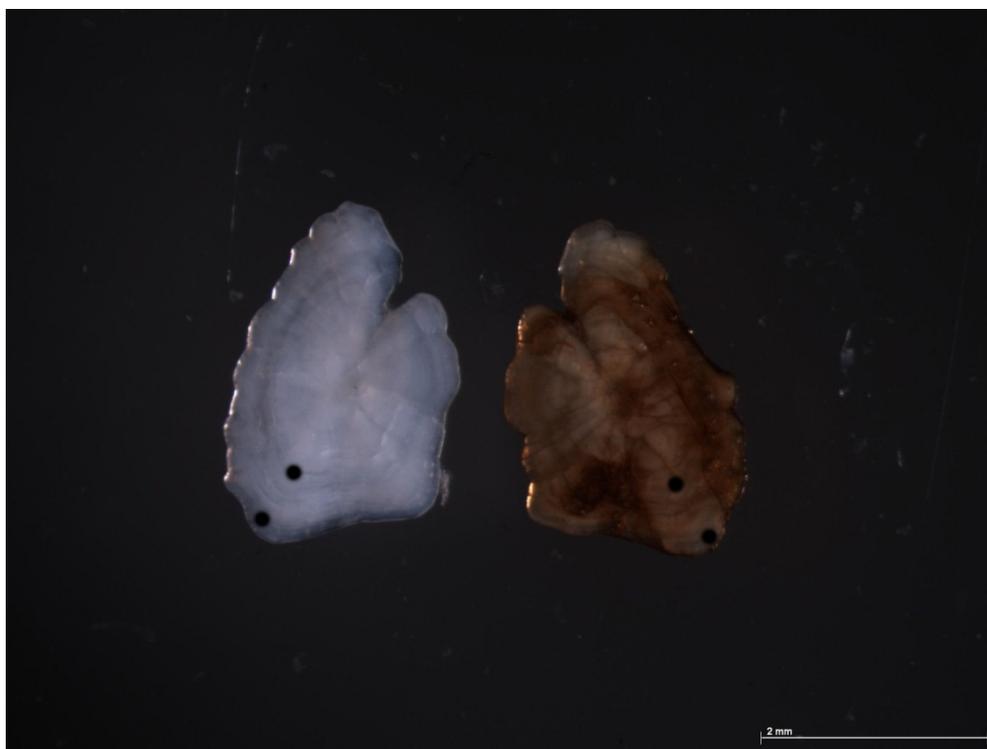


Figure 31: Image of *M. barbatus* (age: 2; length : 195 mm , Sex : F , month of capture : 2) from Balearic islands.

7 Recommended validation methods for *Mullus* spp. ageing from otolith interpretation

For many analytical stock assessments, lack of validated age data are a major source of uncertainty, which could affect the reliability of the assessment results. There exist several methods to validate age readings of calcified structures (CS). Some are designed to identify what constitutes a particular seasonal zone, some to confirm the regularity of deposition of seasonal zones (i.e. marginal increment analysis, marginal analysis, marking CS with oxytetracycline) and some to validate the total age of the structure (marking and re-capture, rearing in captivity, length back-calculated compared with length frequency distribution). An overview of methods and their merits can be found in Campana (2001) and WKNARC (ICES, 2011b) identified review of available studies.

In the framework of the WKCAM2 a problem of identification of the first growth ring has been pointed out. So, validation methods independent of annual growth ring interpretation should be realized:

7.1 Direct methods

So far, there has not been any tagging experiment for *Mullus* spp. neither in the Atlantic nor in the Mediterranean Sea. We suggest a first attempt of tagging during existing experimental surveys (i.e. MEDITS). This should be considered a first step to evaluate the suitability of using tagging experiments for this species.

7.2 Indirect methods

From available length frequency distribution data, on a monthly basis, (i.e. DCR commercial fisheries monitoring of landed and discarded catch), growth parameters could be obtained by a direct fit of length frequency data analysis (i.e. ELEFAN). These results will be compared with those obtained by means of fitting a von Bertalanffy curve to the length-age data from otoliths readings. Besides, from length frequency distributions from annual surveys, modal decomposition analysis (i.e. Bhattacharya's) would be used to break down length frequencies into different modes. Then, using the existing biological knowledge of *Mullus* spp. species, i.e. period of reproduction and recruitment of juvenile individuals to fisheries, it might be possible to "assign" an age to the modal components identified. These results can be compared to the lengths obtained from back-calculation or from the length-age key obtained from otolith readings.

Daily increments studies are advised to validate age 0 individuals and to identify the first annual ring.

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Annex 1: List of participants

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Annex 2: Agenda

Monday 02/07/12

16.00–18.30: introduction, logistics, time schedule and assigning responsibilities.

17.30–18.30: visit of sclerochronology laboratory

Tuesday 03/07/12

09.00–10.30: ToR a Review the results of the new exchanges and compare with those of the previous workshop

10.30–10.45: coffee break

10.45–12.00: ToR a Review the results of the new exchanges and compare with those of the previous workshop

12.00–13.30: lunch

13.30–16.00: ToR b Clarify the interpretation of annual rings and use various validation methods

16.00–16.15: coffee break

16.15–17.15: Review the interpretation of annual rings and reference collection of well defined otoliths

17.15–18.30: Reread of exchange otoliths

Wednesday 04/07/12

09.00–10.30: Reread of exchange otoliths

10.30–10.45: coffee break

10.45–12.00: Reread of exchange otoliths

12.00–13.30: lunch

13.30–16.15: Reread of exchange otoliths (Red mullet)

16.15–16.30: coffee break

16.30–17.30: Analysis of second reading results

17.30–18.30: Visit of Nausicaa

Thursday 05/07/12

09.00–10.30: Draft report

10.30–10.45: coffee break

10.45–12.00: ToR b and c

Guidelines for the interpretation of growth structures in otoliths

12.00–13.30: lunch

13.30–16.00 : ToR d

16.00–16.15 : coffee break

16.15–18.30: Draft reports, Recommendations

Annex 3: WKACM3 terms of reference for the next meeting

The Workshop on Age reading red mullet (*Mullus barbatus*) and striped red mullet (*Mullus surmuletus*) 3 (WKACM3), co-chaired by Francesc Ordines, Spain and Kélig Mahé, France will meet in Palma de Mallorca, Spain, April 2015 to:

- a) Review of available data on the first growth ring through validation studies
- b) Clarify the interpretation of annual growth rings by sex through image analysis (measurements of ring distances and back calculation).
- c) Agreement on guidelines and common ageing criteria.
- d) Increase existing reference collections of otoliths and improve the existing database of otolith images.
- e) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

WKACM3 will report by DATE to the attention of ACOM.

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 which have not been validated. There is necessary to continue to clarify this guideline of age interpretation especially in the Mediterranean sea for <i>Mullus</i> species. Therefore, an appropriate otolith exchange programme will carry out in 2014 for the purpose of inter-calibration between ageing labs. Results of this otolith exchange will discuss during WKACM3. |
| 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 DCR, 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 |
| Linkages to other committees or groups: | PGCCDBS and PGMed |
| Linkages to other organizations: | There is a direct link with the EU DCF. |

Annex 4: Recommendations

| RECOMMENDATION | ADRESSED TO |
|---------------------------------------------------------------------------------------|------------------------------|
| 1.WKACM3 Workshop in 2015 | PGCCDBS, PGMed, ACOM |
| 2. Age validation study to solve the growth rings interpretation | PGCCDBS, PGMed, ACOM, WGNEW |
| 3.Otoliths Exchange of <i>M. surmuletus</i> and <i>barbatus</i> in 2014 | PGCCDBS, PGMed, ACOM, WGNEW |
| 4.Clarify guideline of ageing criteria (e.g.. date of birth) in the Mediterranean sea | PGCCDBS, PGMed, ACOM, WGNARC |

Annex 5: Exchange results of *Mullus* species

In the age bias plots below the mean age recorded ± 2 stdev 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.

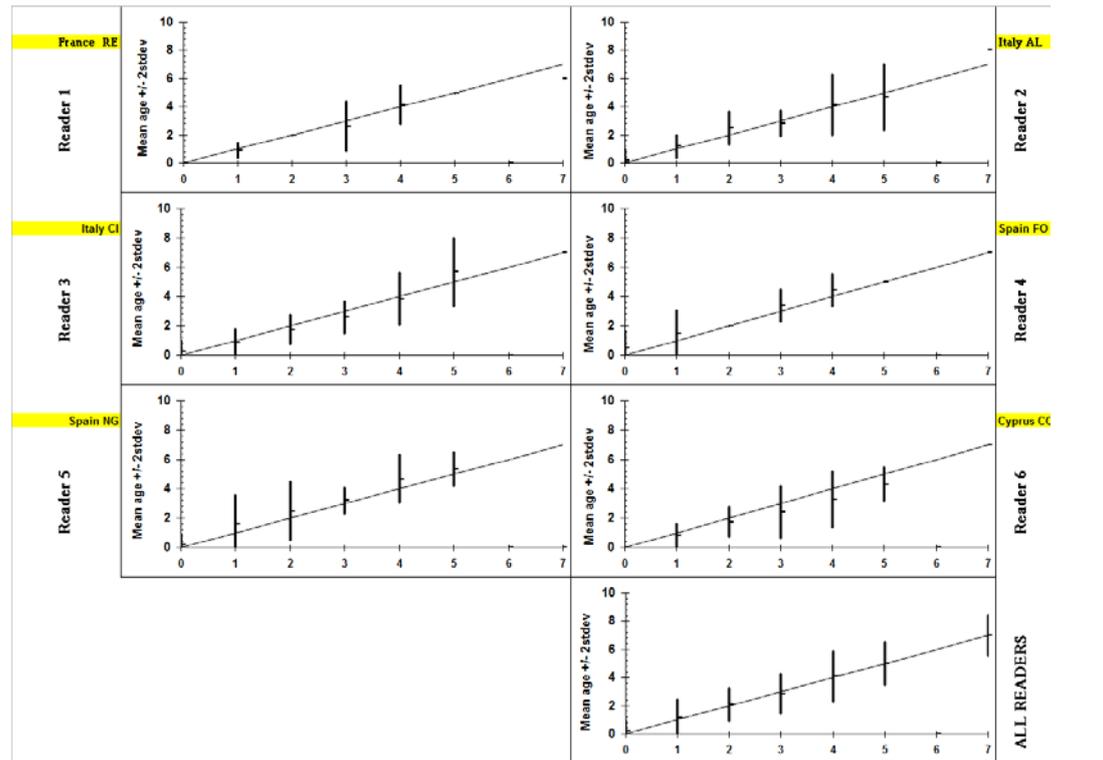


Figure 1: *Mullus surmuletus*, bay of Biscay

The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

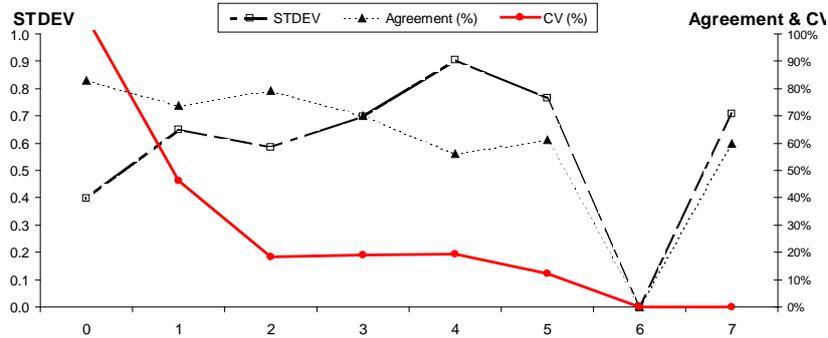


Figure 2: *Mullus surmuletus*, bay of Biscay

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

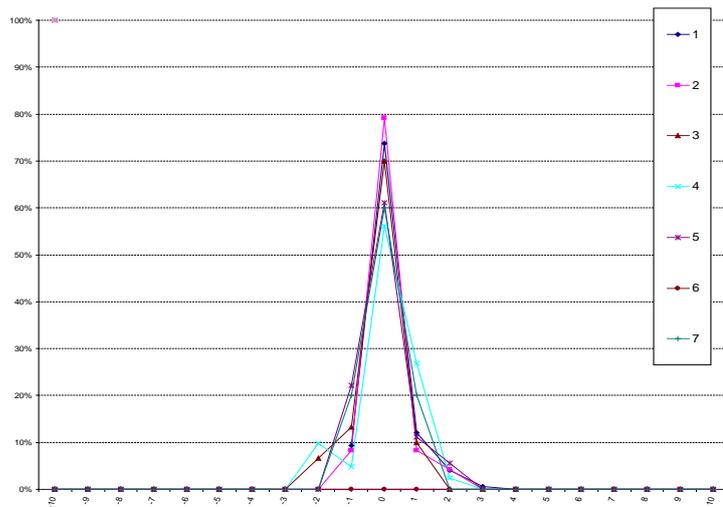


Figure 3: *Mullus surmuletus*, bay of Biscay

In the age bias plots below the mean age recorded ± 2 stdev 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.

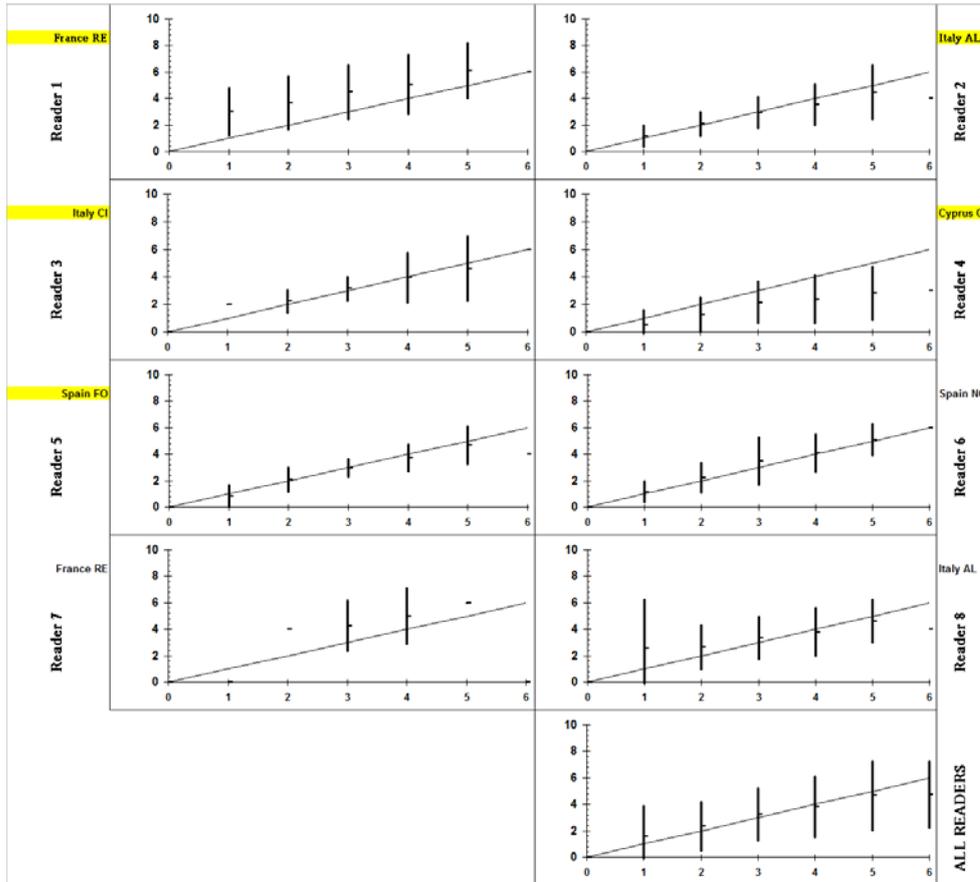


Figure 4: *Mullus surmuletus*, Balearic Islands

The coefficient of variation (CV %), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

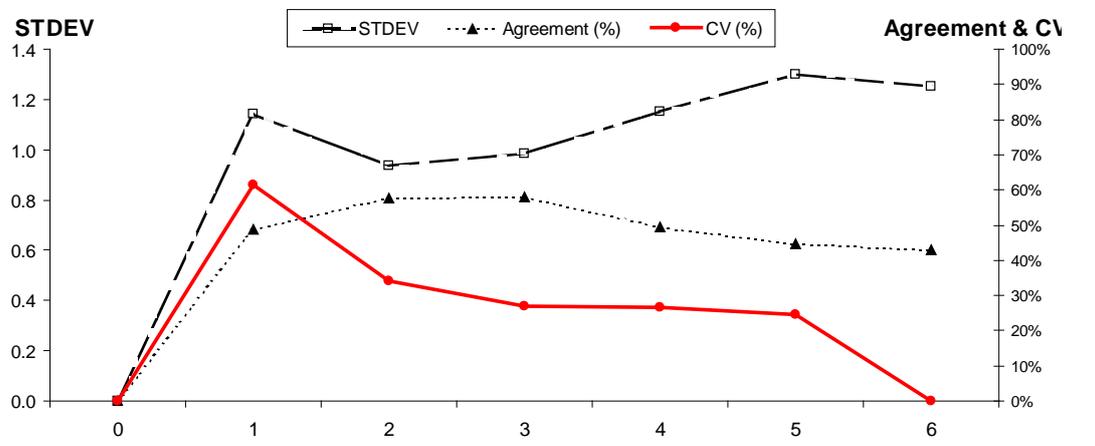


Figure 5: *Mullus surmuletus*, Balearic Islands

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

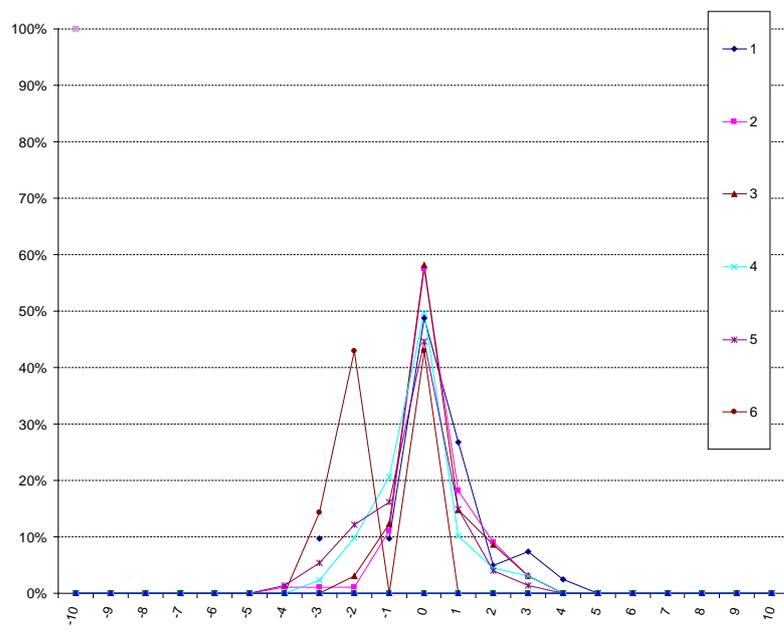


Figure 6: *Mullus surmuletus*, Balearic Islands

In the age bias plots below the mean age recorded ± 2 stdev 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.

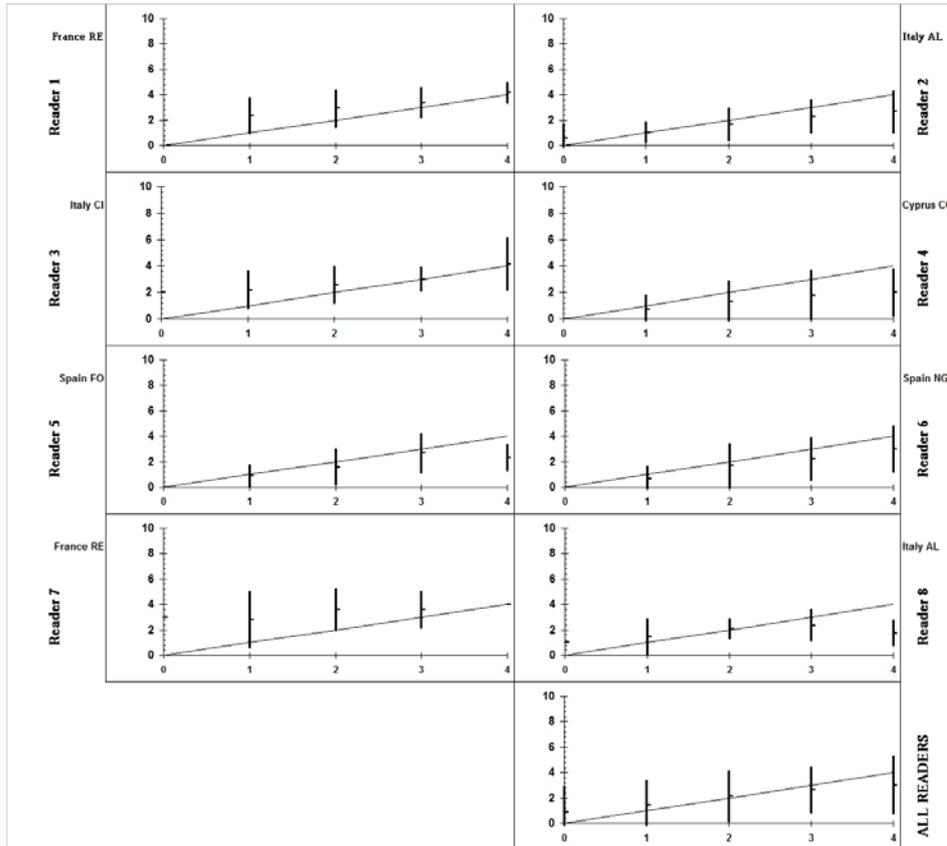


Figure 7: *Mullus barbatus*, southern Spain

The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

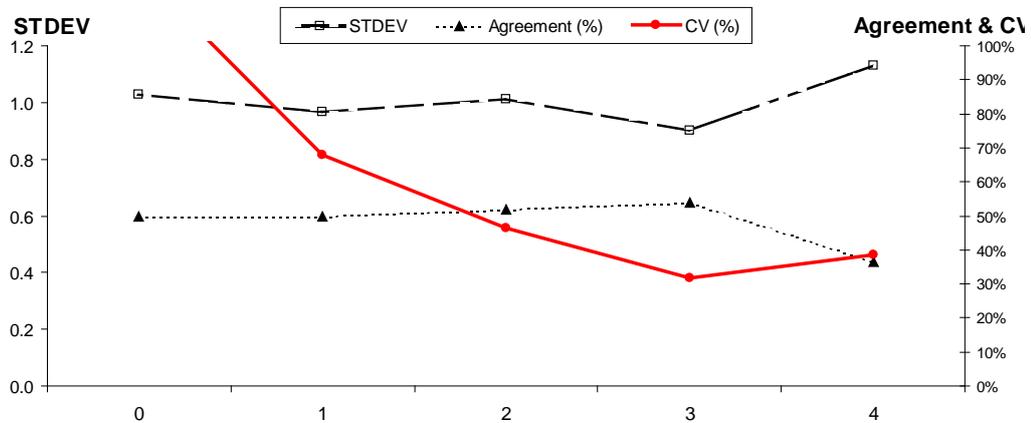


Figure 8: *Mullus barbatus*, southern Spain

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

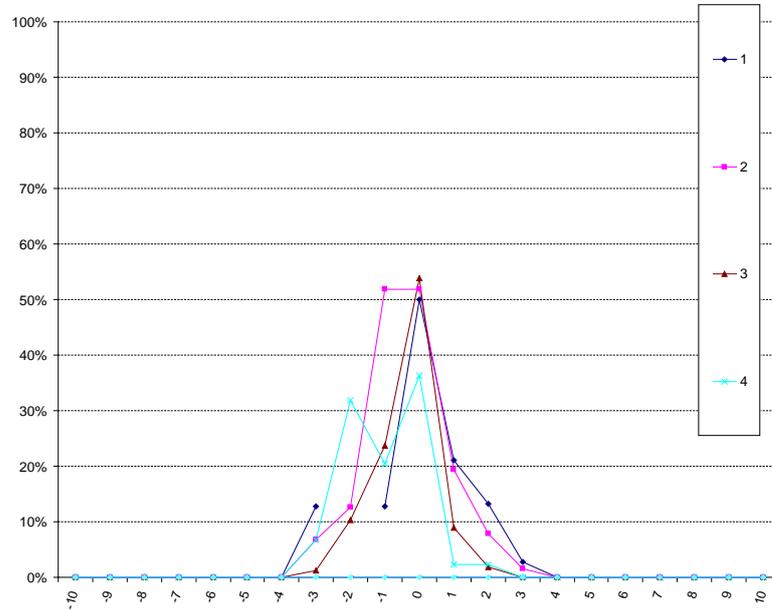


Figure 9: *Mullus barbatus*, southern Spain

In the age bias plots below the mean age recorded ± 2 stdev 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.

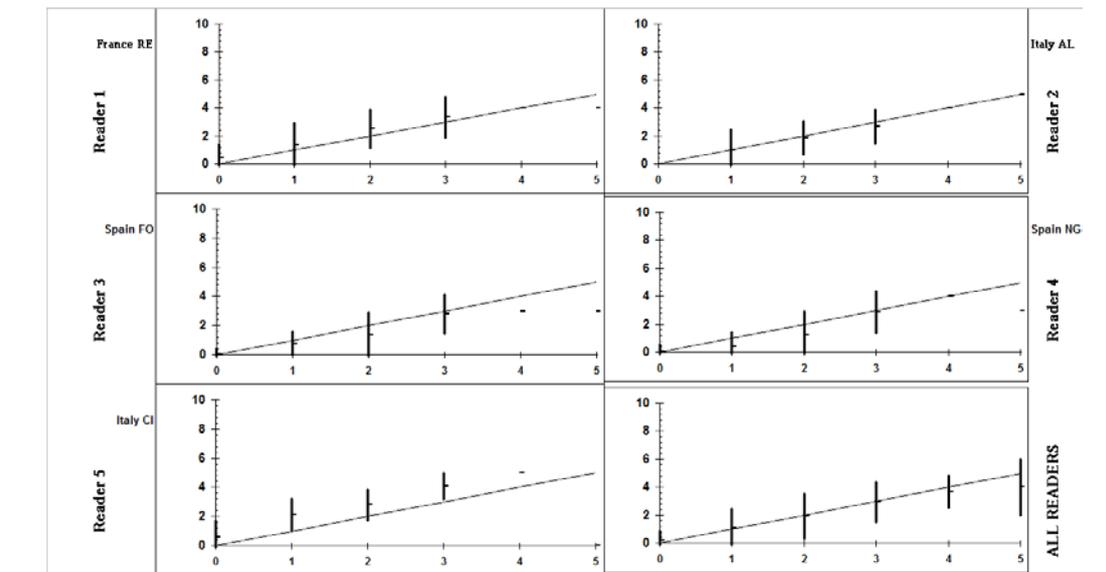


Figure 10: *Mullus barbatus*, southern Adriatic

The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

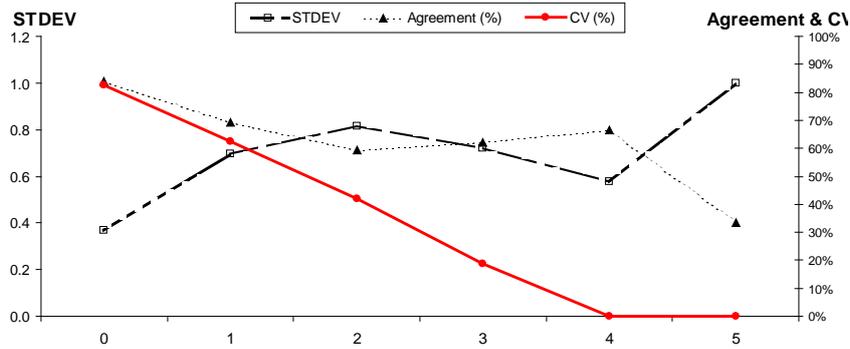


Figure 11: *Mullus barbatus*, southern Adriatic

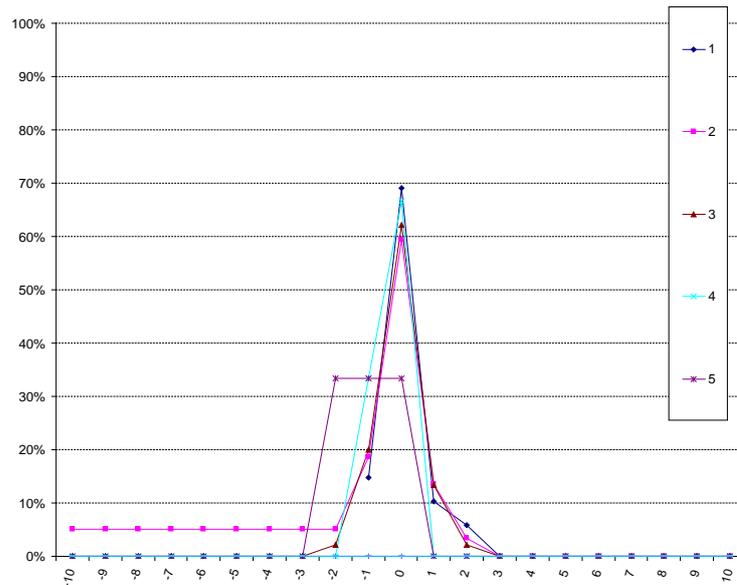


Figure 12: *Mullus barbatus*, southern Adriatic

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

Annex 6: Workshop results of *Mullus* species

In the age bias plots below the mean age recorded \pm 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.

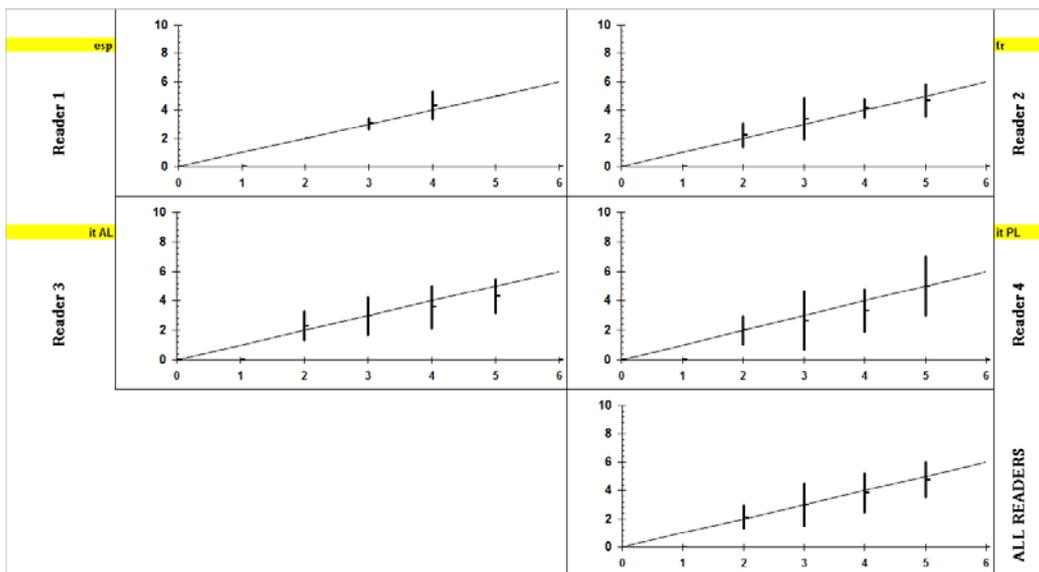


Figure 1: *Mullus surmuletus*, Balearic Islands

The coefficient of variation (CV %), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

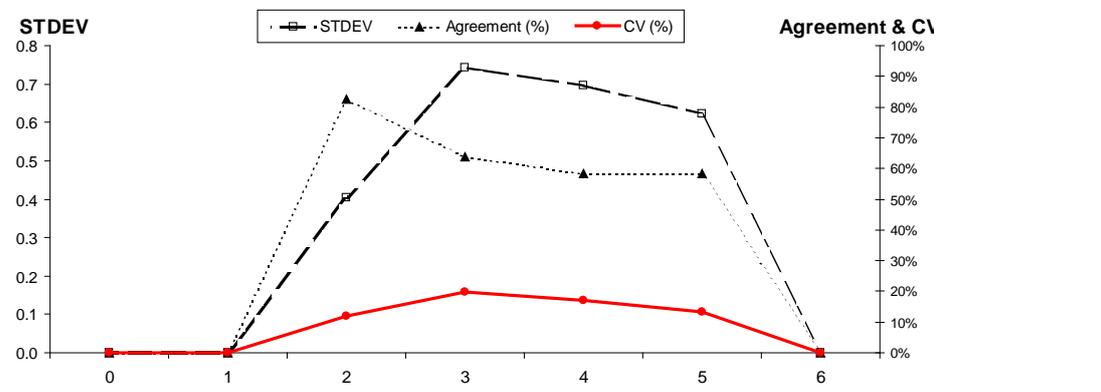


Figure 2: *Mullus surmuletus*, Balearic Islands

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

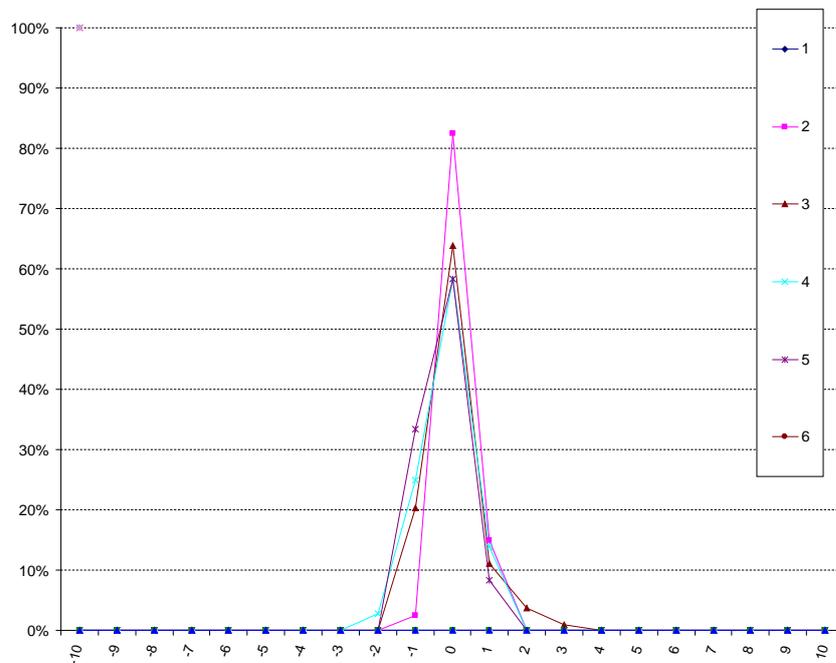


Figure 3: *Mullus surmuletus*, Balearic Islands

In the age bias plots below the mean age recorded ± 2 stdev 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.

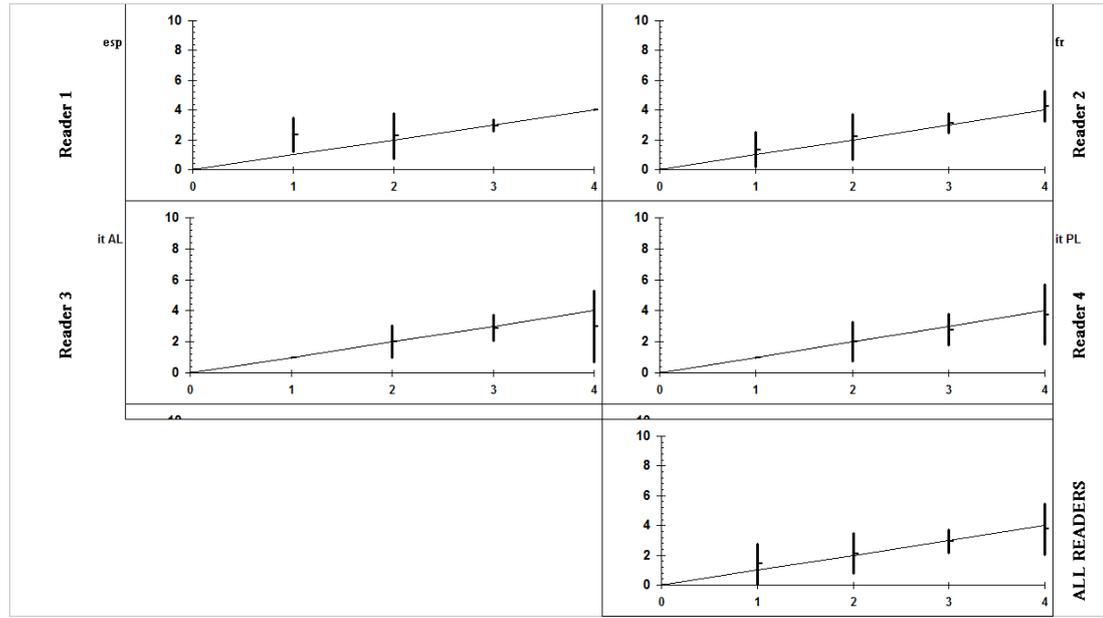


Figure 4: *Mullus barbatus*, southern Spain

The coefficient of variation (CV %), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent 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.

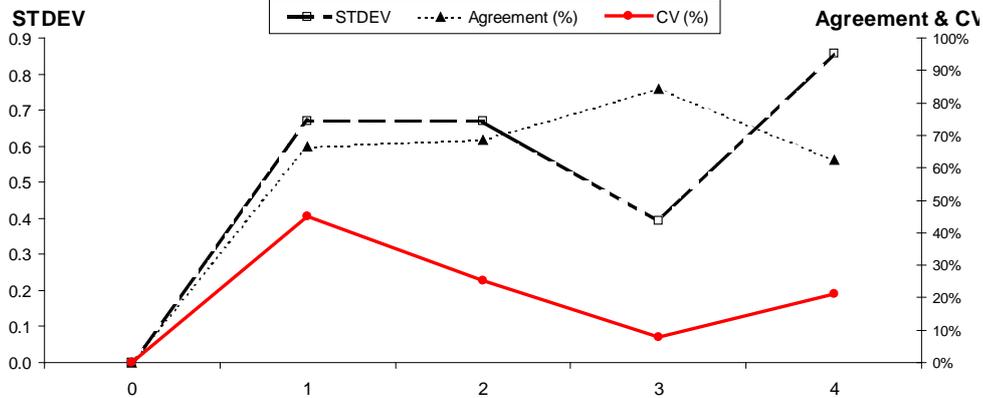


Figure 5: *Mullus barbatus*, southern Spain

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. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

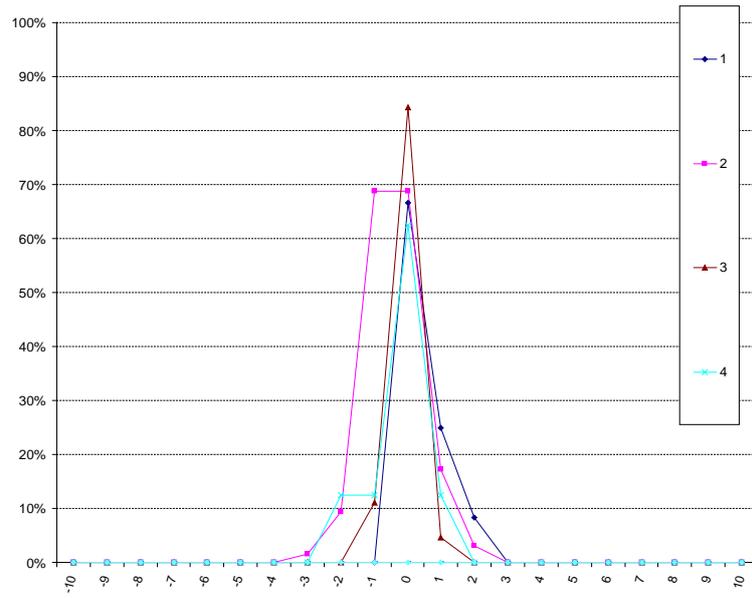


Figure 6: *Mullus barbatus*, southern Spain