Age determination procedures on small and medium pelagic species in Spanish Institute of Oceanography (IEO)

Villamor, B[1]., Navarro, M.R[1]., Hernández, C[1]., Riveiro, I[2]., Meixide, M[2]., Landa, J[1]., Dueñas, C[1]., Antolínez, A[1]., Barrado, J[1]., Peleteiro, M.E[2]., Otero, R[2]., Loureiro, I[2]., López, E[2].

INSTITUTO ESPAÑOL DE OCEANOGRAFÍA 1914

1. Instituto Español de Oceanografía, Centro Oceanográfico de Santander, Promontorio de San Martín s/n, 39080 Santander, Spain; 2. Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Subida a Radio Faro, 50, 36390 Vigo, Spain; Correspondence to B. Villamor: tel: +34 942 291716; fax: +34 942 275072; e-mail: begona.villamor@st.ieo.es.

Recommended format for purposes of citation:

Villamor, B, Navarro, M.R., Hernández, C, Riveiro, I., Meixide, M., Landa, J., Dueñas, C., Antolinez, A., Barrado, J., Peleteiro, M.E., Otero, R., Loureiro, I., Lopez, E. 2014. Age determination procedures on small pelagic species in Spanish Institute of Oceanography (IEO). Int. Doc. IEO, 40 pp.

Abstract

This handbook presents a summary of the age estimation procedures used in Spanish Institute of Oceanography (IEO) for some of the main commercial small and medium pelagic species of the Spanish fleet: anchovy (*Engraulis encrasicolus*), sardine (*Sardina pichardus*), mackerel (*Scomber scombrus*), chuck mackerel (*Scomber colias*), horse mackerel (*Trachurus trachurus*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and blue whiting (*Micromesistius poutassou*). It provides information about the sampling program, otolith extraction and preparation, and the age estimation criteria. A summary of the information related to the age accuracy, validation and corroboration of each species is also presented, as well as that related to the age precision, quality control and verification.

CONTENTS

INTRODUCTION

Since 1970s, the age of small and medium pelagic fish has been determined on the basis of calcified structures (CS), mainly otoliths (*sagittae*), within the Spanish Institute of Oceanography/Instituto Español de Oceanografía (IEO). Currently, the following North Atlantic pelagic fish species are dealt within the institute: anchovy, sardine, mackerel, chub mackerel, horse mackerel, Mediterranean horse mackerel and blue whiting.

For monitoring fish populations, biological samplings are performed throughout the year. These samplings are carried out once a month or quarter, depending on the stock and species. The samples usually come from the fish market (fish landed from the commercial fleet). Also, biological samplings are performed during research surveys. A large number of calcified structures (otoliths) are taken during the annual IEO research surveys carried out in Northeast Atlantic acoustic surveys (PELACUS) and bottom trawl surveys (DEMERSALES, PORCUPINE); and from triennial ichthyoplankton surveys (CAREVA, JUREVA, SAREVA). Some years, depending on the sampling program or research project, the CS are also taken by observers on board of commercial vessels.

Annual age estimations are mainly used in assessment and also in growth studies. In the last decades, they are also used to study the ecology of some species, such as anchovy, sardine and mackerel. For this, daily growth studies have been performed.

At present, six technicians are carrying out the age determination of these small and medium pelagic species in the institute. These age readers participate in the international otolith age reading exchanges and workshops.

Otoliths of small and medium pelagic species are sampled by IEO within the EU data collection framework (DCF). Detailed manuals of each processing step (sampling on commercial vessels, sectioning of otoliths, otoliths storage, mounting the otoliths on resin, age estimation criteria, etc.) are available in Spanish.

1 ANCHOVY (*ENGRAULIS ENCRASICOLUS*)

1.1 SAMPLING PROGRAM FOR AGE ESTIMATION

1.1.1 ANNUAL AGE

Samplings for age determination of anchovy are performed in IEO from the late 70s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 1.1.1.1

Table 1.1.1.1. Number of anchovy otoliths by stock collected from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
Bay of Biscay (Subarea VIII)	1337	497	1834
Division IXa (IXa North)	90	99	189
Total	**1427**	**596**	**2023**

1.1.2 DAILY AGE

During the years between 2006 and 2009, juvenile anchovy otoliths were collected from research surveys for daily growth studies and otolith microstructure analysis. (Table 1.1.2.1)

Table 1.1.2.1 Number of juvenile anchovy otoliths of the Bay of Biscay stock (Subarea VIII) collected for daily growth studies from research surveys during 2006-2009.

Year	Research surveys
2006	897
2007	1010
2008	788
2009	1303
Total	**3998**

1.2 OTOLITH EXTRACTION AND STORAGE

Anchovy otoliths are easy to extract from the fish by cutting through the top of the head (Figure 1.2.1; Figure 1.2.2). It is important to cut very carefully to avoid the otoliths damage as they are very fragile. The organic residues are very carefully removed with tweezers from the otoliths. Then, the otoliths are rinsed with distilled water and stored in black plastic plates with cover.

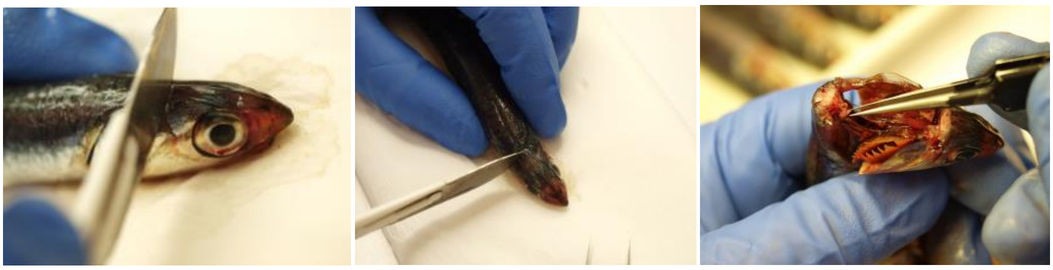

Figure 1.2.1 Extraction of anchovy otolith

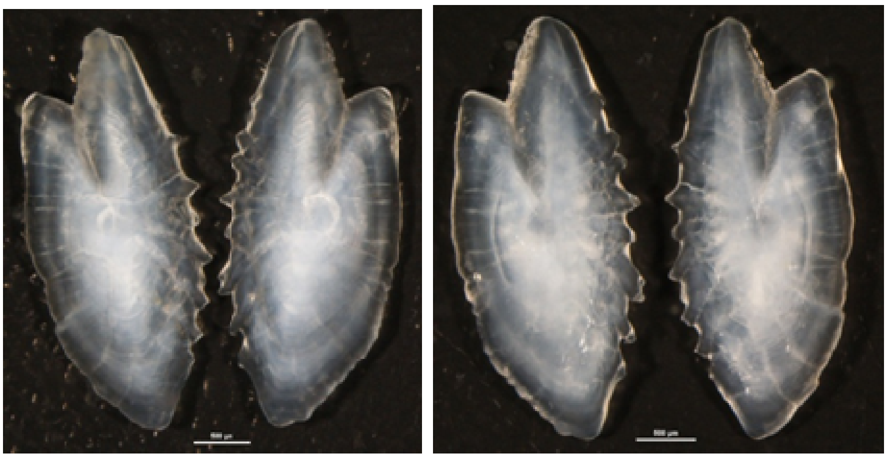

Figure 1.2.2. Anchovy otoliths: Ventral and dorsal view of a specimen of 170 mm of total length

1.3 OTOLITH PREPARATION METHOD

1.3.1 ANNUAL AGE

Like most small pelagic species, anchovy otoliths are read under a binocular microscope on black plastic plates, mounted on non-plastic transparent resin (Eukitt). In recent years, they are also read submerged in water.

1.3.2 DAILY AGE

For daily growth studies of juvenile anchovy, an otolith section is read. In the case of larvae, whole otoliths are read.

The sagitta otoliths extracted from juveniles are cut into sections by a sanding and polishing process (Secor et al., 1992). Each otolith section is processed on the sagittal plane with respect to the fish.

1.4 AGE ESTIMATION METHOD

1.4.1 ANNUAL AGE

Observation: Binocular microscope. The age is also estimated using digital images in some cases, for biometric measures.

Illumination: Reflected light (using fiber optic illuminators),

Magnification: Between 20x and 40x magnification, depending on the otolith size.

Reading axes: Translucent annuli (hyaline) are counted, preferably in the anterior (*rostrum*) and posterior (*post-rostrum*) part of the otolith.

Age estimation criteria: Anchovy age estimation criteria were recommended by ICES 2009. The method was adopted explicitly in the Workshop of anchovy age in 2002 (Uriarte *et al*., 2002) based in the validation study of Uriarte (2002). The method is based on the knowledge of the standard pattern of annual growth in anchovy otoliths, the process of edge formation, and the most common false growth increments (*checks*) which are expected to be found. A set of an opaque and hyaline zone corresponds to an annual growth zone (annulus). The date of birth is conventionally assumed to be the 1st of January and the fish is assigned to a year class on this basis (if an otolith is collected during the first semester the age group correspond to the number of hyaline zones, if the otolith is collected from a fish caught during the second semester, the hyaline edge will not be considered).

Interpretation difficulties: These difficulties could be explained by: 1) the first annulus position; 2) the otolith edge identification (opaque or hyaline); 3) the presence of false growth increments (checks).

1.4.2 DAILY AGE

Observation: Otolith sections viewed through a microscope connected to an image analyzer

Magnification: In larvae, the whole otolith is observed at x1000 magnification along the maximum growth axis; in juveniles, the otolith nucleus is observed at x1000 magnification. The rest of the otolith is observed by two ways, depending on the reading criteria used (see that section): if the GBR criterion is applied, the otolith is observed at x100 or x200 magnification; if the IMR criterion is followed, x400 magnification is used. Close to the otolith margin, at the beginning of the formation of the first translucent zone, the magnification used is x630.

Reading axes: the age estimation is performed along the post-rostrum axis, which is generally the maximum growth axis in sagittal otoliths. The otolith radius is measured along the post-rostrum axis. Otolith microincrements are counted starting in the hatch check until the last. The first evident increment corresponds to the hatch check, at a distance from the primordium between 3.5 and 5 µm (Aldanondo *et al*., 2008).

Age estimation criteria: For daily increment interpretation, two different criteria have been suggested: using the known as group band reading (GBR) criterion, the reader counts every repetitive cyclic set of growth bands (usually two, but occasionally more) as single daily increment, assuming that they are sub-daily marks. And, in the other criterion, known as individual mark reading (IMR), each increment, regardless of its appearance, is counted as single daily increment. According to Cermeño *et al*. (2008), the GBR criterion is the most reliable method for ageing European anchovy. In the Bay of Biscay, the agreement was to apply this methodology (GBR) for anchovy, irrespective of the season and geographical area (Morales-Nin *et al*., 2010; SARDONE protocol, 2010; ICES, 2013a).

Interpretation difficulties: These difficulties could be explain by: 1) difficulties in the interpretation of subdaily increments, double structures or band zones; 2) unclear images, in which is difficult to interpret correctly the daily growth pattern due to under-or over-polishing, poor image acquisition or calibration problems. It is very important to obtain clear images to interpret properly daily micro increments in this species.

1.5 AGE ACCURACY, VALIDATION AND CORROBORATION

1.5.1 ANNUAL AGE

Validation of an absolute age is equivalent to determining the accuracy of age estimation. The distinction between validating the periodicity of the growth increment formation and the absolute age is important. Marginal increment analysis is the most commonly used of the validation methods, and it is used for validating the periodicity of growth increment formation (Campana, 2001). Edge analysis does not assign a state of completion to the marginal increment, but rather records its presence as either an opaque or translucent zone (Campana, 2001).

The periodicity of growth increment formation in anchovy has been validated by edge analysis in the Bay of Biscay (Uriarte, 2002a). The season in which the hyaline annuli are formed was determined by the examination of the frequency of the distribution of otolith edge types throughout the year. The hyaline edge is mostly predominant from October to March-April while the opaque edge predominates in the rest of the year.

Age corroboration methods are not equivalent to those of age validation, since corroboratory methods support or are correlated with a particular ageing method, but are not directly or logically linked (Campana, 2001). The corroboration method of tracking strong/weak year-classes compares the interval between yearly samples and the increase in the apparent modal age of a recruitment pulse as determined through annulus counts (Campana, 2001). This method, also considered as an "indirect validation" method, indicates that an age-reading method is accurate if the age composition of exceptionally good or weak year classes can be tracked over a long period of time (Panfili *et al*., 2002).

The age estimation criteria of anchovy were corroborated (or indirectly validated) by tracking year-classes abundance indices 1982-1989 in research surveys in the Bay of Biscay (ICES, 2002; Uriarte *et al*., 2002).

Based on different daily growth studies, the position of the first annulus was validated and the position of the first false ring or check was corroborated in anchovy in the Bay of Biscay (ICES, 2013a). Annual increment deposition in the otoliths of young-of-the-year European anchovy was validated (Aldanondo *et al*., 2013). Early anchovy juveniles were maintained in captivity from October 2012 until April 2013 and the first annulus was validated using daily increments counts. According to that, the first opaque band is completed in October-November, whereas the translucent band is formed by March-April. As reference point, the first annulus would be at 1156 (+- 70 µm). The first hyaline ring (check) and all hyaline rings that are at a distance less than 850 µm (± 100µm) should be considered as a check (Hernandez *et al*., 2013).

1.5.1 DAILY AGE

The daily periodicity of micro-increment deposition was validated in early life stages of anchovy, though in a few areas of distribution. As far as anchovy is concerned, validation studies were carried out exclusively in individuals from Bay of Biscay. In particular, the daily increment deposition was validated in hatched eggs and larvae reared in the laboratory under different temperature conditions (Aldanondo *et al*., 2008), as well as in wild juveniles marked by immersion in oxytetracycline hydrochloride (OTC) and reared until reaching adulthood over a period of 2 years (Cermeño *et al*., 2003).

1.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

1.6.1 ANNUAL AGE

Verification confirms the consistency of the age interpretation, i.e. the repeatability and/or precision of a numerical interpretation that may be independent of the age. Considerable efforts are made by international committees to standardize the age interpretations (Panfili *et al*., 2002). ICES WKNARC-2 (ICES, 2013b) recommended internal (within each research institute) and external (among institutes) quality controls to confirm the consistency of the age interpretation.

Anchovy age estimation criteria used by IEO readers have been externally verified by international otoliths exchanges and workshops (Astudillo *et al*., 1990; Villamor and Uriarte, 1996; Uriarte, 2002a; Uriarte *et al*., 2002, 2006 and 2007; ICES, 2009). Current IEO age readers have participated in recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (ICES, 2009).

The same otolith is read by two readers, or else, by one experienced reader, but then, each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations coincide. When a discrepancy between them is found, a third reading is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended in WKNARC-1 (ICES, 2011a). Three possible results of age quality (AQ) are distinguished:

- AQ1. Otoliths easy to age whose estimated age is assigned without any doubt at the first reading. The estimated age is considered as the final age for that individual;

- AQ2. Otoliths difficult to age, whose estimated age is assigned with certain doubts at the first reading and are examined a second time. If this second age estimation is the same as in the first, it is assigned as the final age of the individual. If doubts between the two estimations still remained, the otolith is read a third time, and the most frequent age of the three values is assigned as the final age, or else, the age is left with two values (eg 5/4), being the first one considered as more confident age.

- AQ3. Otoliths practically unreadable or very difficult to age, with doubts among three or more possible age values. Those otoliths are excluded from further analysis.

1.6.2 DAILY AGE

To test the quality control of daily age estimations, internal (reading procedure) and external (planned otoliths exchanges and working groups) practices (Sardone, 2008; ICES, 2013a) are developed by IEO. The internal practice concerns mainly to repeated readings performed independently by one or more readers, to check the precision in age estimations. Generally, otoliths are discarded when the reading precision shows an error higher than 5-10%.

2 SARDINE (*SARDINA PILCHARDUS*)

2.1 SAMPLING PROGRAM FOR AGE ESTIMATION

2.1.1 ANNUAL AGE

Samplings for age determination of sardine are performed in IEO since the early 80s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 2.1.1.1

Table 2.1.1.1. Number of sardine otoliths collected by stock from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
Divisions VIIIc-IXa	3123	301	3424
Division VIIIb	-	80	80
Total	**3123**	**381**	**3504**

2.1.2 DAILY AGE

Daily growth studies in Atlantic Iberian sardine started in the early 90'. Studies were directed to validate daily ring deposition in culture larvae and to estimate birthdates in individuals from natural environments (Alvárez and Butler, 1992; Alemany and Alvárez, 1994; Alvárez and Alemany, 1997; Alvarez, 2005).

In recent years, larvae and juvenile (up to 18 months) of sardina were obtained in captivity from fertilized eggs captured in the wild. Otoliths of known age from these individuals are being currently analyzed.

2.2 OTOLITH EXTRACTION AND STORAGE

Sardine otoliths are easy to extract from the fish by cutting through the top of the head (Figure 2.2.1; Figure 2.2.2). They are less fragile than mackerel or anchovy otoliths and, in comparison, are larger and tend to be exposed after the cut. The organic residues are very carefully removed with tweezers from the otoliths. Then, the otoliths are rinsed with distilled water and stored in black plastic plates with cover.

Figure 2.2.1. Extraction of sardine otolith.

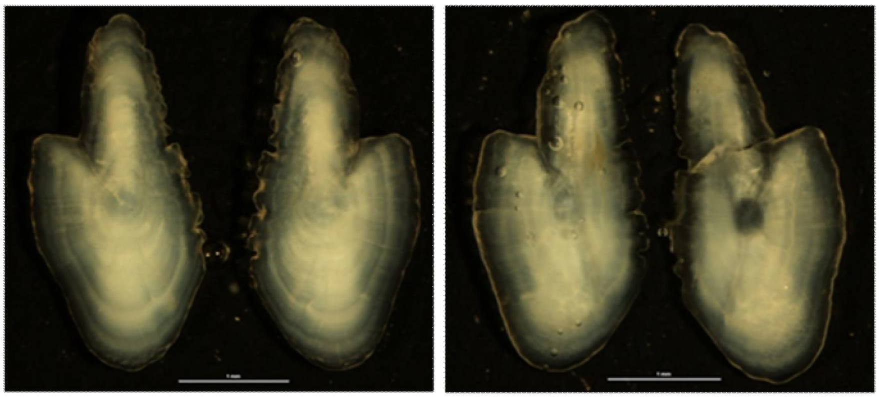

Figure 2.2.2. Sardine otoliths: Ventral and dorsal view

2.3 OTOLITH PREPARATION METHOD

2.3.1 ANNUAL AGE

Like most small pelagic species, sardine otoliths are read under a binocular microscope on black plastic plates, mounted on transparent resin.

2.3.2 DAILY AGE

For daily growth studies of juvenile sardine, an otolith section is read. In the case of larvae, whole otoliths are read.

The sagitta otoliths extracted from juveniles and pre-recruits are cut into sections by a sanding and polishing process (Secor *et al*., 1992). Each otolith section is processed on the sagittal plane with respect to the fish.

2.4 AGE ESTIMATION METHOD

2.4.1 ANNUAL AGE

Observation: Binocular microscope. Also, in some cases and for biometric measures, the age is estimated using digital images.

Illumination: Reflected light (using fiber optic illuminators),

Magnification: Using 20x magnification. The magnification can be increased near the otolith edge to improve the discrimination of narrow rings in older individuals.

Reading axes: Translucent annuli are counted and the edge type is determined in the posterior (post-rostrum) otolith region, where annuli are generally clearer and the otolith growth is higher.

Age estimation criteria: The sardine age estimation criteria were recommended in last ICES sardine age reading workshop (ICES, 2011b). The method was adopted explicitly in FAO (1979) and can be summarized as follows: i)a set of an opaque and hyaline zone corresponds to an annual growth zone (annulus).ii) the date of birth is conventionally assumed to be the 1st of January and the fish is assigned to a year class on this basis (if an otolith is collected during the first semester the age group correspond to the number of hyaline zones, if the otolith is collected from a fish caught during the second semester, the hyaline edge will not be considered).

Interpretation difficulties: The main discrepancies in sardine age determination are the identification of the otolith edge type and the first annulus. Two problems related to the edge type were discussed at the last workshop: 1) difficulty in identifying the edge type (hyaline or opaque); 2) variation in the seasonality of the edge type.

2.4.2 DAILY AGE

Observation: microscope connected to an image analyzer

Magnification: In larvae, the whole otolith is observed at 1000x magnification along the maximum growth axis. In juveniles, the otolith nucleus is observed at x1000 magnification. The rest of the otolith is observed by two ways depending on the reading criteria (See section 1.4): if the GBR criterion is applied, the otolith is observed at x100 or x200 magnification; if the IMR criterion is followed, x400 magnification is used.

Reading axes: The age estimation is performed along the post-rostrum axis, which is generally the maximum growth axis of sagittal otoliths. The otolith radius is measured along the post-rostrum axis. Otolith micro-increments are counted starting in the hatch check until the last. The first evident increment corresponds to the hatch check, at a distance from the primordium between 5 and 7 µm (Alemany and Alvarez, 1994).

Age estimation criteria: For daily increment interpretation of sardine otoliths, the same recommendations suggested for anchovy by ICES 2013a (ICES WKMIAS) are followed (See section 1.4). In the Bay of Biscay and Atlantic Iberian Peninsula, the agreement was to apply the GBR criteria for sardine, irrespective of the season and geographical area (Morales-Nin et al., 2010; SARDONE protocol, 2010; ICES, 2013a).

Interpretation difficulties: These difficulties could be explain by: 1) difficulties in the interpretation of sub-daily increments, double structures or band zones; 2) unclear images, in which is difficult to interpret correctly the daily growth pattern due to under-or over-polishing, poor image acquisition or calibration problems. It is very important to obtain clear images to interpret properly daily micro-increments in this species.

2.5 AGE ACCURACY, VALIDATION AND CORROBORATION

2.5.1 ANNUAL AGE

It is suggested to measure the first annulus and all the translucent annuli laid down before a distance from the primordium of less than 1000 µm should be considered as checks (Silva *et al.,* 2012). That is based on i) the validation of daily annulus formation in sardine larvae and juveniles (Alemany and Alvarez, 1994), and ii) the corroboration of the position of the false annual ring (check) formed before its first winter annulus, through the micro-increment counts (ICES, 2011b; ICES, 2013a).

2.5.2 DAILY AGE

The daily periodicity of micro-increments deposition was validated in early life stages of sardine in a few areas of distribution. In the Atlantic Iberian area, the daily deposition was validated in sagitta otoliths of wild sardine larvae reared from birth until complete the absorption of the yolk sac (Re, 1984; Alemany and Alvarez, 1994). Similarly, validation of daily increment formation of otoliths was carried out using a mesocosm experiment in sardine larvae grown on natural environmental conditions in the Adriatic Sea (Panfili, 2012).

2.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

2.6.1 ANNUAL AGE

Sardine age estimation criteria used by IEO readers have been externally verified by international otoliths exchanges and workshops (ICES, 2005; ICES, 2011b). Current IEO age readers have participated in recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (ICES, 2011b).

The same otolith is read by two readers, or else, by one experienced reader, but then, each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations coincide. When a discrepancy between them is found, a third reading is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1.

2.6.2 DAILY AGE

To test the quality control of daily age estimations, internal (reading procedure) and external (planned otoliths exchanges and working groups) practices (SARDONE, 2008; ICES, 2013a) are developed by IEO. The internal practice concerned mainly to repeated readings performed independently by one or more readers, to check the precision in age estimation. Generally, otoliths are discarded when the error in reading precision is higher than 5-10%.

3 MACKEREL (*SCOMBER SCOMBRUS*)

3.1 SAMPLING PROGRAM FOR AGE ESTIMATION

3.1.1 ANNUAL AGE

Samplings for age determination of mackerel are performed in IEO since 1982, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 3.1.1.1

Table 3.1.1.1. Number of mackerel otoliths collected by stock from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
Southern Component (Divisions VIIIc,IXa)	1032	1548	2580
Western Component (Divisions VIIIab, VII)	0	11	11
Total	**1032**	**1559**	**2591**

3.1.2 DAILY AGE

In 2000, otoliths of larvae, post-larvae and juveniles of mackerel were collected from research surveys and commercial fleet, for daily growth studies and otolith microstructure analysis within the SEAMAR Project (Table 3.1.2.1).

Table 3.1.2.1 Number of mackerel otoliths collected for daily growth studies from the commercial fleet and research surveys in 2000.

Stock	Commercial fleet	Research surveys	Total
Southern component (VIIIc,IXa)- Larvae	-	1126	1126
Southern Component (VIIIc,IXa)- Juveniles	160	218	378
Total	**160**	**1344**	**1504**

3.2 OTOLITH EXTRACTION AND STORAGE

Mackerel otoliths are more difficult to extract from the fish than in other small pelagic species, because they are very small compared to the fish. They are extracted by cutting through the top of the head (Figure 3.2.1; Fig. 3.2.2). It is important to cut very carefully to avoid the otoliths damage as they are very fragile. The organic residues are very carefully removed with tweezers from the otoliths. Then, the otoliths are rinsed with distilled water and stored in black plastic plates with cover.

Figure 3.2.1. Extraction of mackerel otolith

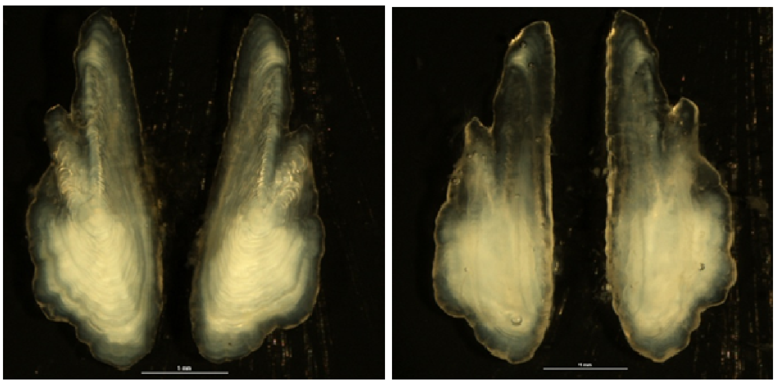

Figure 3.2.2. Mackerel otoliths: Ventral and dorsal view

3.3 OTOLITH PREPARATION METHOD

3.4.1 ANNUAL AGE

Like most small pelagic species, mackerel otoliths are read under a binocular microscope on black plastic plates, mounted on non-plastic transparent resin (Eukitt).

3.4.2 DAILY AGE

In daily growth studies of juvenile mackerel, an otolith section is read. In the case of larvae, whole otoliths are read.

The sagitta otoliths are extracted from larvae and postlarvae using fine dissection needles under a binocular microscope 3-5 months after they have been preserved. Then, they are washed with distilled water, dried and mounted on glass slides within transparent synthetic enamel (Secor *et al.*, 1992). Larvae and postlarvae otoliths are mounted whole and with the concave side upwards.

The sagitta otoliths extracted from juveniles and pre-recruits are cut into sections by a sanding and polishing process (Secor *et al.*, 1992). Each otolith section is processed on the sagittal plane with respect to the fish.

3.4 AGE ESTIMATION METHOD

3.4.1 ANNUAL AGE

Observation: Binocular microscope. The age is also estimated using digital images in some cases, for biometric measures.

Illumination: Reflected light (using fiber optic illuminators).

Magnification: Between 20x and 40x magnification, depending on the otolith size.

Reading axes: Translucent annuli (hyaline) are counted, preferably in the anterior (*rostrum*) and posterior (*post-rostrum*) part of the otolith. When different ages are recorded in these two otolith areas, the older one is then considered (ICES, 1995).

Age estimation criteria: Mackerel age estimation criteria were recommended by ICES (1995;2010). The method was adopted explicitly in the Workshop of mackerel age in 1995 (ICES, 1995) based in the age validation of this species, by reading otoliths of known age (obtained from a tagging program).

The date of birth is conventionally assumed to be the 1st of January and the fish is assigned to a year class on this basis (if an otolith is collected during the first semester the age group correspond to the number of hyaline zones, if the otolith is collected from a fish caught during the second semester, the hyaline edge will not be considered).

Interpretation difficulties: These difficulties could be explained by: 1) different length of time for the opaque zone formation of the otolith between the different areas during the first year; 2) otolith edge interpretation; 3) possible presence of false annuli associated with the first maturity; 4) slowdown growth in older fish to such an extent that the opaque and translucent (hyaline) zones become confused and are more difficult to distinguish.

3.4.2 DAILY AGE

Observation: Whole otoliths of larvae and otoliths sections of juvenile mackerels viewed through a microscope connected to a personal computer via a video camera.

Magnification: Otoliths are examined at 1000x. Growth increments are counted and measured using image analysis software (VISILOG/TNPC 3.1). In order to estimate the growth increments of both larvae otoliths and juveniles otolith sections, the objective (x100) is used with immersion oil.

Reading axes: Larvae otoliths are spherical, so that they can be read in any axis. However, otoliths sections of juveniles are processed along the short axis, and the growth increments are counted along the dorsal-ventral axis, as described by D'Amours *et al.* (1990). The numbering of growth increments on the otoliths is carried out within two triangular surfaces pointing towards the core on the dorsal-ventral axis relative to the fish; these two surfaces are defined as the standard reading fields, which correspond to the short axis of the otolith (Figure 3.4.2.1).

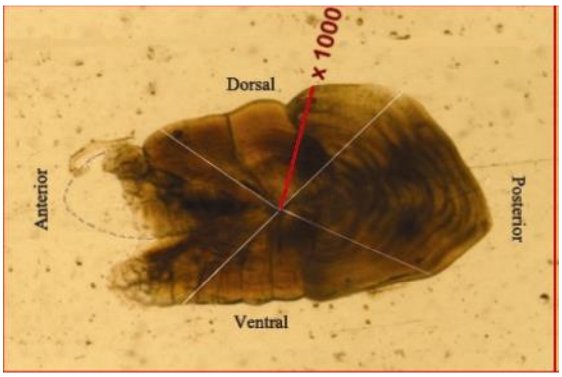

Figure 3.4.2.1. Sagittal section (at 5x) of mackerel sagitta otolith (164 mm standard length), showing: the standard fields of the growth rings interpretation in two triangular surfaces (blank lines) from the center of the otolith, in dorsal-ventral direction and the area (red line) that is processed in the image analyzer at 1000 magnification.

Age estimation criteria: The numbering of otolith growth increments begins at the hatch check. The last increment is omitted because it is considered incomplete since it does not represent a full day. The deposition of daily growth increments in mackerel larvae, post-larvae and juveniles has been validated by Migoya (1989) and D'Amours *et al*. (1990) in the Northwest Atlantic, and by Mendiola and Alvarez (2008) in the Northeast Atlantic.

Interpretation difficulties: These difficulties could be explained by: 1) difficulties in the interpretation of subdaily increments, double structures or band zones; 2) difficulties in the interpretation of intermediate areas without growth increments in juvenile otoliths; 3) unclear images, in which is difficult to interpret correctly the daily growth pattern, due to under- or over-polishing, poor image acquisition or calibration problems. It is very important to obtain clear images to interpret properly daily micro-increments in this species.

3.5 AGE ACCURACY, VALIDATION AND CORROBORATION

3.5.1 ANNUAL AGE

The existing material of such work is rather limited, particularly the one related to the actual yearly age structures of mackerel otoliths. The validation of North East Atlantic mackerel annual age criteria was established in 1995 to age 11, using fish of known age, which were determined by mark-recapture experiments (ICES, 1995). Older ages could not be validated by this method and it was assumed that the modal age of the age assignments performed by readers for the same otolith corresponded to the actual age, as recommended by ICES (1994).

3.5.2 DAILY AGE

The deposition of daily growth rings in larvae, post-larvae and juveniles of mackerel was validated by Migoya (1989) and D'Amours *et al.* (1990), in Northwest Atlantic, and by Mendiola and Alvarez (2008) in Northeast Atlantic. Direct transformation of the number of increments in age (days) is well justified.

Migoya (1989) and Mendiola and Alvarez (2008) incubated mackerel eggs in the laboratory and showed that the deposit of the first increment in the otolith occurred on the hatching day and that the increments were formed daily. In addition, D'Amours et al. (1990) performed a validation experiment on mackerel juveniles in captivity, marking their otoliths with a fluorescent substance and showing that the increments were deposited on a daily basis.

3.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

3.6.1 ANNUAL AGE

Mackerel age estimation criteria used by IEO readers have been externally verified by international otoliths exchanges and workshops (Villamor and Meixide, 1994; Anon., 1995; SAMFISH, 2002; ICES, 2010). Current IEO age readers have participated in recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (ICES, 2010).

The same otolith is read by two readers, or else, by one experienced reader, but then, each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, a third reading is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1 of this document.

3.6.2 DAILY AGE

To test the quality control of daily age estimations, internal (reading procedure) and external (planned otoliths exchanges and working groups) practices (SEAMAR, 2002) are developed by IEO. The internal practice concerned mainly to repeated readings performed independently by one or more readers, to check the precision in age estimation. Generally, otoliths are discarded when the error in reading precision is higher than 5-10%.

4. CHUB MACKEREL (*SCOMBER COLIAS*)

4.1 SAMPLING PROGRAM FOR AGE ESTIMATION

Routine samplings for age determination of chub mackerel began to be performed in IEO in 2011, as a new requirement of the DCF for the period 2011-2013. Samples are obtained from both, commercial catches and research surveys (Table 4.1.1) from Division VIIIc and Subdivision IXa North.

Table 4.1.1. Number of chub mackerel otoliths collected from the commercial fleet and research surveys in 2012.

Areas	Commercial fleet	Research surveys	Total
Division VIIIc	1049	246	1295
Division IXa North	1112	-	1112
Total	**2161**	**246**	**2407**

4.2 OTOLITH EXTRACTION AND STORAGE

Chub mackerel otoliths (Figure 4.2.1) are extracted and storage in the same way as the mackerel ones (see section 3.2)

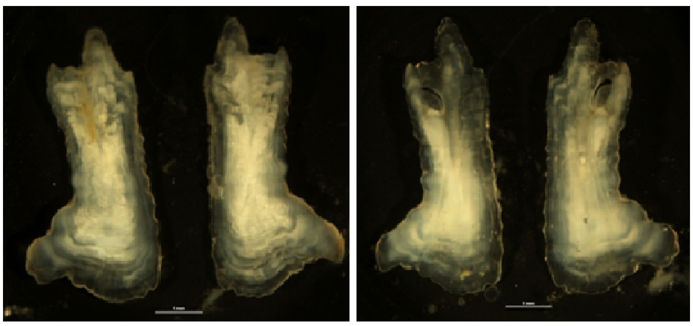

Figure 4.2.1. Chub Mackerel otoliths: dorsal and ventral view.

4.3 OTOLITH PREPARATION METHOD

Like most small pelagic species, chub mackerel otoliths are read under a binocular microscope on black plastic plates, mounted on non-plastic transparent resin (Eukitt).

4.4 AGE ESTIMATION METHOD

Observation: Binocular microscope. The age is also estimated using digital images in some cases, for biometric measures

Illumination: Reflected light (using fiber optic illuminators).

Magnification: Between 20x and 40x magnification, depending on the otolith size.

Reading axes: Translucent rings (hyaline) are counted, preferably in the anterior (*rostrum*) and posterior (*post-rostrum*) part of the otolith.

Age estimation criteria: The criteria for the age determination of *Scomber colias* are still developing. Due to the similarity between both species, and while the criteria for the age determination of chub mackerel are not fully defined, to follow the reading criteria applied to mackerel is recommended (see section 3.4.1.). Although certain peculiarities are observed, such as: 1) higher presence of false growth increments; 2) priority is given to the post-rostrum in the age interpretation, as annuli tend to be better determined in this area than in the rest of the otolith; 3) usually, the otolith point provides little help in the age interpretation in chub mackerel, since the annuli are very crowded in this area and are difficult to determine, especially in otoliths of older specimens.

Interpretation difficulties: These difficulties could be explained by: 1) difficulties in identifying the first annulus; 2) difficulties in differentiating between true annual rings (annuli) and false rings (checks); 3) insufficient annual growth pattern recognition; and 4) insufficient criterion regarding the otolith edge that can be expected to be seen along the year.

4.5 AGE ACCURACY, VALIDATION AND CORROBORATION

The available material of such work is rather limited. Currently, studies are underway to validate the age determination criteria of this species (semi-direct validation studies).

4.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

Only one otolith exchange of chub mackerel has been carried out recently (Martins *et al.*, 2014), where the IEO reader have participated, showing good values of agreement, precision and relative accuracy.

Chub mackerel otoliths are read by a specific experienced reader. Each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, a third estimation is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1 of this document.

5 HORSE MACKEREL (*TRACHURUS TRACHURUS*)

5.1 SAMPLING PROGRAM FOR AGE ESTIMATION

Samplings for age determination of horse mackerel are carried out in IEO since 1980s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 5.1.1.

Table 5.1.1. Number of horse mackerel otoliths collected by stock from the commercial fleet and research surveys in 2012.

Stock	Commercial fleet	Research surveys	Total
Southern Stock (IXa North)	583	240	823
Western Stock (VIIIc-VIIIb)	1515	1220	2735
Total	**2098**	**1460**	**3558**

5.2 OTOLITH EXTRACTION AND STORAGE

Horse mackerel otoliths can be easily extracted by cutting through the top of the head, at the operculum level (Figure 5.2.1, Figure 5.2.2). They are large and robust, so they are not easily broken during the extraction. The organic residues are removed from the otoliths with tweezers. Then, the otoliths are rinsed with distilled water. Nowadays, horse mackerel otoliths are stored in micro-tubes, but until 2012, they were stored dry in envelopes.

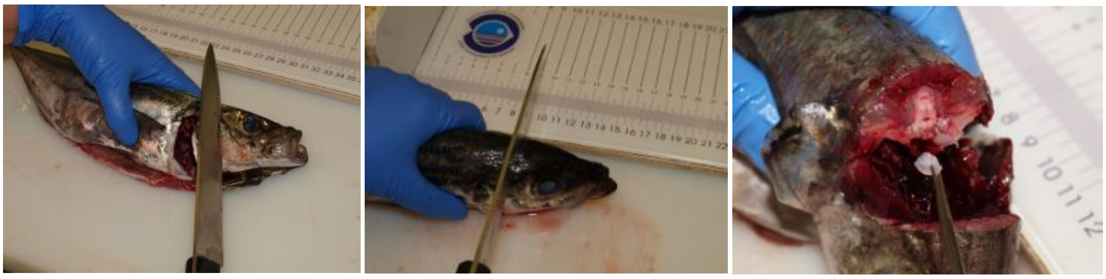

Figure 5.2.1. Extraction of horse mackerel otolith

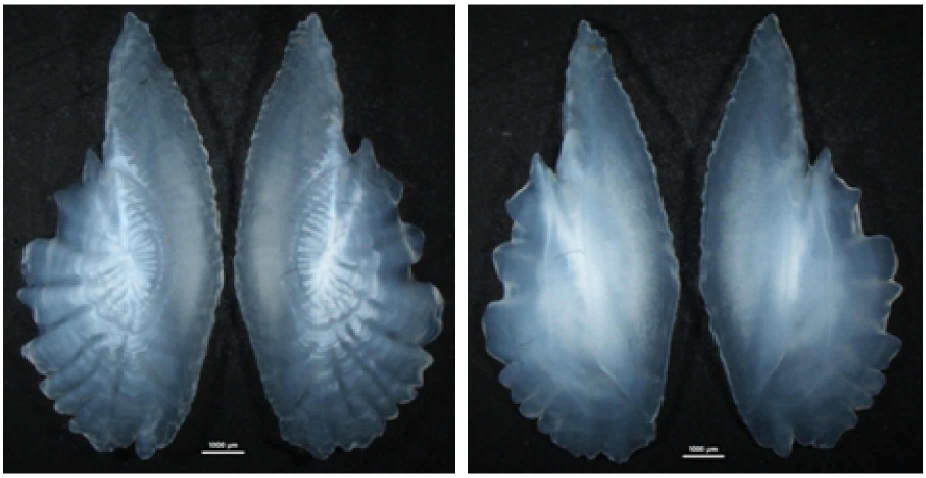

Figure 5.2.2. Horse mackerel otoliths: dorsal and ventral view.

5.3 OTOLITH PREPARATION METHOD

Horse mackerel otoliths are very thick, which prevent a correct observation of its structural characteristics; therefore it is necessary to make an adequate preparation. In the past, otoliths were burned, but at present, they are cut in sections. The objective of the otolith cutting process is to obtain thin sections of them. Usually, horse mackerel otoliths are cut when the fish size is over 26 cm; below this size, the otoliths are usually read directly whole. The use of otolith sections is mandatory with older ages (6-8).

Whole Otoliths: *Trachurus trachurus and Trachurus mediterraneus* <25 cm: Otoliths, after being extracted from each sampled fish, are stored in microtubes. They are placed in a recipient with alcohol and glycerin, to be observed under a binocular microscope.

Sectioned Otoliths: *Trachurus trachurus and Trachurus mediterraneus* >25cm: The whole otoliths are embedded in polyester resin in an aluminum 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. The resulting sections are stuck in glass slides and properly labeled.

5.4 AGE ESTIMATION METHOD

Observation: The sectioned otoliths are placed in a recipient with alcohol and glycerin and examined by a binocular microscope. Also, in some cases and for biometric measures, the age is estimated on digital images.

Illumination: Reflected light (using fiber optic illuminators).

Magnification: Between 15x and 50x magnification, depending on the otolith size. The examination method depends on the technique of preparation used. So, whole otoliths of young fishes are observed with low magnification (between 15x and 25x), otherwise, false rings may confuse inexperienced readers. Otoliths sections of older fishes are examined with a magnification between 30x and 50x.

Reading axes: Translucent rings (hyaline) are counted, preferably in the anterior (*rostrum*) and posterior (*post-rostrum*) parts of the otolith.

Age estimation criteria: Horse mackerel age estimation criteria were established by ICES (1999; and 2012), based on direct age validation studies (Kerstan and Waldrom, 1995) and on indirect validation studies (ICES, 1999; Waldron and Kerstan, 2001; Abaunza *et al*., 2003).

Interpretation difficulties: In general, the age of horse mackerel otoliths is very difficult to estimate in older fishes because they become thick with age. The first annuli interpretation in both, young and older fishes, appears to be the major cause of differences. The dissimilarity of the false rings and the variety of the true annuli make difficult to follow the true annuli formation. In some otoliths, problems are caused by the otoliths edge interpretation.

5.5 AGE ACCURACY, VALIDATION AND CORROBORATION

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

Waldron and Kerstan (2001) used two methods to validate age determination of horse mackerel otoliths. In the first, whole otoliths were examined; age was determined by identifying and counting annuli and marginal increment widths were also measured and served to estimate ages, which ranged from 0.6–4.3 years. In the second method, otoliths were examined with a scanning electron microscope and fish ages were determined by daily increment counts. Estimated ages agreed with ages derived by counting daily increments, thus validating the ages of horse mackerel up to four years.

5.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

Horse mackerel age estimation criteria used by IEO readers have been externally verified by international otoliths exchanges and workshops (ICES, 1999; Bolle *et al*., 2006; ICES, 2012). Current IEO age readers have participated in recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (ICES, 2012).

The same otolith is read by two readers, or else, by one experienced reader, but then, each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, a third reading is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1 of this document.

6 MEDITERRANEAN HORSE MACKEREL (*TRACHURUS MEDITERRANEUS*)

6.1 SAMPLING PROGRAM FOR AGE ESTIMATION

Like in the case of chub mackerel, the sampling routine for the age determination of Mediterranean horse mackerel began to be performed in IEO in 2011, as a new requirement of the DCF for the period 2011-2013. Samples are taken from both, commercial catches and research surveys (Table 6.1.1.1), mainly from Division VIIIc.

Tabla 6.1.1.1. Number of Mediterranean horse mackerel otoliths collected from the commercial fleet and research surveys in 2012.

Areas	Commercial fleet	Research surveys	Total
Division IXa North	1	-	1
Division VIIIb	-	6	6
Division VIIIc	422	196	618
Total	**423**	**202**	**625**

6.2 OTOLITH EXTRACTION AND STORAGE

It is the same as in the case of horse mackerel (see section 5.2).

6.3 OTOLITH PREPARATION METHOD

It is applied the same as in the case of horse mackerel otolith (see section 5.3).

6.4 AGE ESTIMATION METHOD

The same protocol as in the case of horse mackerel is followed to estimate the age of Mediterranean horse mackerel.

Observation, illumination, magnification and reading axes are the same as in horse mackerel (see section 5.4)

Age estimation criteria: They were recommended by ICES (2012), as in the case of horse mackerel.

Interpretation difficulties: Mediterranean horse mackerel otoliths are difficult to interpret, in a similar way as in horse mackerel otoliths, whose age determination for older individuals is particularly imprecise. However, Mediterranean horse mackerel otoliths present specific problems when assigning ages to younger individuals, related to the first annulus interpretation.

6.5 AGE ACCURACY, VALIDATION AND CORROBORATION

Accuracy cannot be evaluated in the Atlantic area since validation data are not available at the moment and the true age determination in this species is not possible. However, in the Eastern Mediterranean the time of annulus completion was estimated by the study of monthly marginal increments (Karlou-Riga, 2000).

6.6 AGE PRECISION, VERIFICATION AND QUALITY CONTROL

Only one otolith exchange and workshop of Mediterranean horse mackerel have been carried out recently (ICES, 2012), where the current IEO reader has participated.

Mediterranean horse mackerel otoliths are read by a specific experienced reader. Each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, a third estimation is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1 of this document.

7 BLUE WHITING (*MICROMESISTIUS POUTASSOU*)

7.1 SAMPLING PROGRAM FOR AGE ESTIMATION

Samplings for age determination of blue whiting are carried out in IEO since 1980s, from both, commercial catches and research surveys. The number of otoliths collected in 2012 is shown in Table 7.1.1

Table 7.1.1. Number of blue whiting otoliths collected from the commercial fleet and research surveys in 2012.

Areas	Commercial fleet	Research surveys	Total
Division VIIIc	629	1722	2351
Division IXa North		275	275
Total	**629**	**1997**	**2626**

7.2 OTOLITH EXTRACTION AND STORAGE

Blue whiting otoliths can be easily extracted by cutting through the top of the head. They are large and robust, so they are not easily broken during the extraction. Once the otoliths are extracted (Figure 7.2.1; Figure 7.2.2), they are rubbed with the fingers to eliminate the remains of organic material and washed with water. Then, they are stored dry in microtubes. Until 2012, the otoliths were stored dry in envelopes and also in the past, the otoliths were stored in distilled water with thymol.

Figure 7.2.1 Extraction of blue whiting otolith

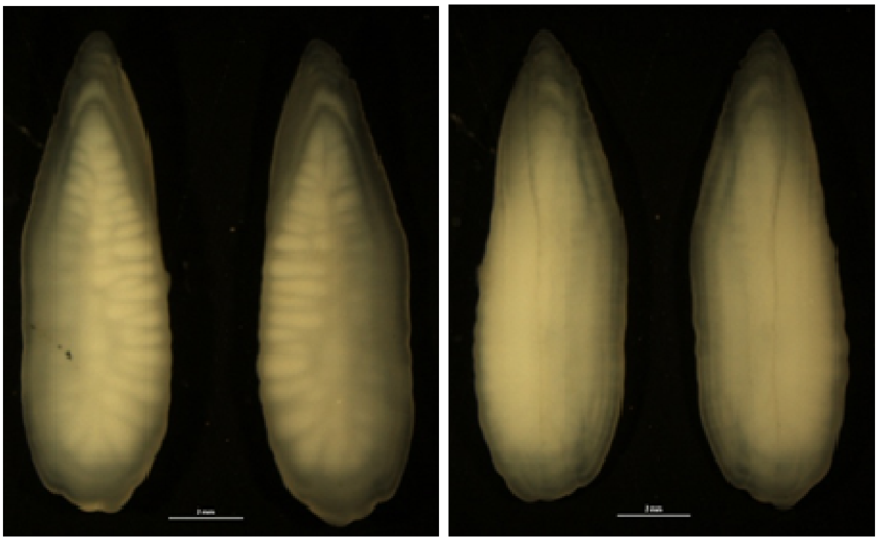

Figure 7.2.2 Whiting otoliths: dorsal and ventral view.

7.3 OTOLITH PREPARATION METHOD

Blue whiting otoliths are read whole, under a binocular microscope, immersed in water. In the past, these otoliths were mounted on a block of resin and cut in sections.

It is recommended to read the blue whiting otoliths immediately after their removal from the fish. To store the otoliths dry, but being soaked in water for 24 hours beforehand is also recommended. If the otoliths are stored in water longer than 7 days, the shape/composition of the otolith seems to change (due to unstable pH of the water), so the storage is recommended to be done dry. The otolith should not be soaked in water for more than 48 hours each time, as it could possibly affect the annuli structure due to the freshwater composition.

7. 4 AGE ESTIMATION METHOD

<u>Observation</u>: Binocular microscope.

<u>Ilumination</u>: Reflected light (using fiber optic illuminators),

Magnification: Using 6/6.4x magnifications against a black background where 12 e.p.u (eyepiece units) are equal to 2 mm. Amplification and light intensity can be adjusted by each reader. The magnification is increased with the otolith size.

Reading axis: Blue whiting otoliths are interpreted by reading the translucent annuli (hyalines) up the *rostrum* area and using the whole otolith pattern as a guide. Usually, the clearest pattern is seen when the convex side of the otolith is facing down (sulcus side facing down). However, handling the otolith, turning it in various directions, may be a way of assuring the estimated age. With difficult otoliths, to read both (the concave and convex otolith sides) is recommended, to gain a better annuli interpretation.

Age estimation criteria: Blue whiting age estimation criteria were recommended by ICES (2005; 2013). The correct annulus identification can be induced by measuring the inner annulus size. It will thereby be possible to avoid including the Bailey's zone (Bailey, 1970) as the first annulus. Usually, a growth increment in the size range of50 to 56 e.p.u (corresponding: 8.33 to 9.33 mm.) can be considered the first annulus (ICES 2005).

Interpretation difficulties: The interpretation of blue whiting otoliths is generally difficult. Even in well-marked otolith annuli, there are subjective decisions to be made, which are highly dependent on each reader's experience. These difficulties could be explained by: 1) the difficulty in the interpretation of the first annulus position, where the Bower zone is clear; 2) the presence of false and split growth increments, which is a severe problem that causes large differences in age; 3) the edge interpretation, which is another source of error that produces a difference of one year in the assigned age.

7.5 AGE ACCURACY, VALIDATION AND CORROBORATION

Little has been performed to validate the blue whiting age estimations. There is only one study on southern blue whiting (Hanchet and Uozumi, 1996).

7.6 PRECISION, VERIFICATION AND QUALITY CONTROL

Blue whiting age estimation criteria used by the IEO readers have been externally verified by international otoliths exchanges and workshops (Monstad and Linkowski, 1988; Meixide, 1990; Anon, 1993; ICES, 2005; ICES, 2013c). Current IEO age readers have participated in recent otoliths exchanges and workshops, showing good values of agreement, precision and relative accuracy (ICES, 2013c).

Blue whiting otoliths are read by a specific experienced reader. Each otolith is read twice, on two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, a third estimation is carried out. Unreadable otoliths are rejected.

In addition to the age estimation, the quality (or credibility) of each age estimation is also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011a), as it is described for anchovy in section 1.6.1 of this document.

ACKNOWLEDGEMENTS

This work was funded through the Spanish Institute of Oceanography (IEO, BIOPEL project) and European Union (Data Collection Framework Program). We would like to thank all those who collaborated in that project. The authors wish also to thank the IEO scientific teams in CO Santander, CO Vigo y CO A Coruña, as well as all the participants in the IEO research surveys that made the fish samplings. We also thank the samplers in harbors and fish markets, as well as the fishers, crews and ship-owners of commercial vessels, for their collaboration in the fish samplings.

REFERENCES

Abaunza P., Gordo L., Karlou-Riga C., Murta A., Eltink A.T.G.W., García Santamaría M.T., Zimmermann C., Hammer C., Lucio P., Iversen S.A., Molloy J., and Gallo, E. 2003. Growth and reproduction of horse mackerel, Trachurus trachurus (Carangidae). Reviews in Fish Biology and Fisheries, 13(1): 27-61.

Aldanondo, N., Cotano, U., Etxebeste, E., Irigoien, X., Álvarez, P., Martínez de Murguía, A. and Herrero, D.L. 2008. Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. Fish. Res. 93: 257–264.

Alemany, F. and Álvarez, F. 1994. Formation of initial daily increments in sagital otoliths of reared and wild *Sardina pilchardus* yolk-sac larvae. Marine Biology, 121: 35-39.

Alvárez, F. 2005. Crecimiento diario de *Sardina pilchardus* (Walbaum, 1792) (Pisces, Clupeidae) y su aplicación al estudio de procesos de reclutamiento. PhD thesis. 121 pp.

Alvarez, F., and Alemany, F. 1997. Birthdate analysis and its application to the study of recruitment of the Atlanto-Iberian sardine *Sardina pilchardus*. Fish. Bull. 95: 187-194.

Alvarez, F., and Butler, J.L. 1992. First attempt to determine birthdates and environmental relationship of juvenile sardine, *Sardina pilchardus* (Walb.), in the region of Vigo (NW Spain) during 1988. Bol. Inst. Esp. Oceanogr., 8(1): 115-122.

Anon., 1993: Report of the Blue Whiting Otolith Reading Workshop. ICES C.M. 1993/H:6

Anon., 1993. Report of the clupeidos otolith microstructure Workshop. Informe Técnico. Instituto Español de Oceanografía. No 145.

Anon., 1995. Report of the Workshop on Mackerel Otolith Reading. ICES CM 1995/H:1,45pp.

Astudillo, A., Lucio, P., Prouzet, P. and Uriarte, A. 1990. Summary of the results concerning the otolith reading exercise on anchovy held in San Sebastián (Spain) in January 1990. *Working Document to the 1990 ICES Working Group on the assessment of the Stocks of sardine, horse mackerel and anchovy. Copenhagen, 20 - 29 June, 1990*.

Bailey, R.S. 1970. A re-interpretation of age determination in blue whiting *Micromesistius poutassou.* ICES CM 1970/F:3l. 5.

Bolle, L.J., Abaunza, P., Albrecht, C., DijKman-Dilkes, A., Dueñas, C., Gentschouw, G., Gill, H., Holst, G., Moreira, A., Mullins, E., Rico, I., Rijs, S., Smith, T., Thaarup, A., and Ulleweit, J. 2006. Report of the Horse Mackerel Exchange and Workshop 2006. CVO report: 11.007.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Cermeño, P. Morales-Nin, B.and Uriarte, A., 2006. Juvenile European anchovy otolith microstructure. Scientia Marina, 70(3):553-557.

Cermeño, P., Uriarte, A., Morales-Nin, B.,. Cotano, U., and Alvarez, P. 2008. Setting up interpretation criteria for ageing juvenile european anchovy otoliths. Sci. Mar. 72(4)

D'Amours, D., Landry, J.G. and Lambert, T.C.. 1990. Growth of juvenile (0-Group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 47: 2212-2218.

Eltink, A.T.G.W. and Kuiter, C. 1989. Validation of ageing techniques on otoliths of horse mackerel (*Trachurus trachurus* L.).ICES C.M. 1989/H:43, 15 pp.

FAO 1979. Reportof the workshop on the standardization of age reading techniques for *Sardina pilchardus* Walb.). CECAF Tech. Rep., 79/18.

ICES, 1994. Report of the Workshop on sampling strategies for age and maturity. ICES, C.M. 1994/D:1

ICES, 1999. Report of the Horse Mackerel Otolith Workshop, Lowestoft, 15–19 January 1999. ICES, C.M. 1999/G:16, 57 pp.

ICES, 2005a. Workshop on sardine otolith age reading and biology. IPIMAR Lisbon, Portugal 27 June – 1 July, 2005

ICES, 2005b. Report of the Blue Whiting Otolith Ageing Workshop. ICES C.M. 2005/ACFM, Hirt-shals, Denmark 13-16 June 2005.

ICES, 2009. Report of the Workshop on Age reading of European anchovy (WKARA). ICES CM 2009/ACOM:43

ICES, 2010. Report of the Workshop on Age Reading of Mackerel. ICES CM 2010/ACOM: 46

ICES, 2011a. Report of the Workshop of National Age Readings Coordinators (WKNARC), 5-9 September 2011, Boulogne-sur-Mer, France. ICES CM 2011/ACOM:45. 175 pp.

ICES, 2011b. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS). ICES CM 2011/ACOM:42

ICES, 2012. Workshop on Age Reading of horse mackerel, Mediterranean horse mackerel and blue jack mackerel (WKARHOM). ICES CM 2012/ACOM.

ICES, 2013a. Report of the Workshop on Micro increment daily growth in European Anchovy and Sardine (WKMIAS). ICES CM 2013/ACOM

ICES, 2013b. Report of the Second Workshop of National Age Readings Coordinator (WKNARC-2). ICES CM 2013/ACOM: 52

ICES, 2013c. Report of the Workshop on the Age Reading of Blue Whiting (WKARBLUE). ICES CM 2013/ACOM:53.

Karlou-Riga, C.2000. Otolith morphology and age and growth of *Tarchurus mediterraneus* (Steindachner) in the Eastern Mediterranean. Fisheries Research, 46: 69-82

Kerstan, M. and Waldrom, M.E. 1995. Age validation in Horse Mackerel (*Trachurus trachurus*) otoliths from the NE and SE Atlantic Ocean. ICES CM 1995/H:23.

Letaconnoux, R. 1951. Contribution à l'études des espèces de genre Trachurus et spécialement du Trachurus trachurus (Linné 1758) de la côte Belge. Mem. Off. Sci. Techn. Peche Marit. 15, 1–67.

Martins, M.M., Silva, A., Navarro, M.R., Vivas, M., Rodríguez, E., Morais, D. and Villamor. B. 2014. Report of chub mackerel (*Scomber colias*, Gmelin 1798) otolith exchange 2012-2013. WD to PGCCDBS 2014, Horta (Azores, Portugal) 17-21 February 2014. ICES CM 2014/ACOM

Meixide, M. 1990. Results of the Blue Whiting Otolith Exchange. ICES C.M. 1990/H:37

Mendiola, D. and Álvarez, P. 2008. Validation of daily increments in the otolith microstructure of Northeast Atlantic mackerel fish larvae. Fisheries Research 89 (2008): 300-304

Migoya, M.C. 1989. Étude de la croissance des larves de maquereau bleu (*Scomber scombrus*, L.) dans le Golfe du St. Laurent, a partir de l'examen de la microstructure des otoliths. M.Sc.Thesis, Université du Québec à Rimouski, Québec, 84 p.

Monstad, T and Linkowski, T. 1988. On the otolith exchange programme. Working note to the meeting of Blue Whiting Assessment Working Group, Copenhaguen, 21-28 September 1988.

Morales-Nin, B., Aldanondo, N. and Alvarez, I. 2010. Age determination of larval and juvenile small pelagics: the importance of a common protocol. Rapp. Comm. int. Mer Médit., 39, 2010

Panfili, J., de Pontual, H., Troadec, H. and Wright, P. J. 2002. Manual of Fish Sclerochronology. IFREMER-IRD co-edition, Brest. 464 pp.

Panfili, M. 2012. Ecology of early life stages of small pelagic fish Engraulis encrasicolus and Sardina pilchardus in the Adriatic Sea. PhD thesis. Universita Politecnica delle Marche

Ré, P. 1984. Evidence of daily and hourly growth in pilchard larvae based on otolith growth increments, *Sardina pilchardus* (Walbaum, 1792) Cybium, 8 (1): 33–38.

SAMFISH, 2002. Final Report of SAMFISH project: Improving sampling of western and southern european atlantic fisheries. SAMFISH project, Study Contract 99/009.

SARDONE Project. Improving assessement and management of small pelagic species in the Mediterranean. Final Report of the SARDONE project. UE-FP6- 44294

Silva, A.V., Meneses, I., andSilva, A. 2012. Determinacao de idade de juvenis de sardinha (*Sardina pilchardus*, Walbaum 1792) a partir de características morfometricas dos individuos e dos otolitos. Relatorios Científicos e Tecnicos, IPIMAR, no. 53. http://ipimariniap. ipimar.pt/. ISSN 1645-863x

Secor, D.H., Dean, J.M. and Laban, E.H.. 1992. Otolith Removal and Preparation for Microstructural Examination. En D.K. Stevenson, S.E. Campana (eds.). Otolith microstructure examination and analysis, 73-100. Can. Spec. Publ. Fish. Aquat. Sci., 117, 130 p.

Uriarte, A. 2002. 2001 Anchovy Otolith Exchange programme from Subarea VIII and Division IXa. *Annex to PELASSES report- EU study Project -EC DG XIV Contract nº99/010.*

Uriarte, A. 2002a. Descripción y validación de la metodología de determinación de la edad en la anchoa del golfo de Vizcaya mediante el examen de sus otolitos y estudio de su crecimiento anual*. Manuscrito Interno AZTI.*

Uriarte, A., Blanco, M., Cendrero, O., Grellier, P., Millán, M. , Morais, A. and Rico, I. 2002.Report of the Workshop on anchovy otoliths from subarea VIII and division IXa *Annex to PELASSES report EU study Project -EC DG XIV Contract nº99/010 and Working Document to the ICES Working Group on the assessment of Mackerel, Horse Mackerel, Sardine and Anchovy. Copenhagen, 10-19 September 2002*.

Uriarte, A., Blanco, M., Duhamel, E., Grellier, P., Rico, I., and Villamor, B.. 2006. 2005 Anchovy Otolith Exchange Programme from Subarea VIII. *Working Document to the 2006 ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS*).

Uriarte, A., Dueñas, C., Duhamel, E., Grellier, P., Rico1, I. and Villamor B. 2007. 2006 Anchovy Otolith Workshop. *Working Document to the 2007 ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS*).

Villamor, B. and Meixide. M. 1995. Results of the Mackerel (*Scomber scombrus*, L.) otolith exchange programme in 1994. ICES CM 1995/H: 12, 31 p.

Villamor B. and Uriarte, A. 1996. Results of the Anchovy (*Engraulis encrasicolus* L.) exchange programme in 1996. *WD to the 1996 ICES WG on the assessment of mackerel, horse mackerel, sardine and anchovy. Copenhagen, 13-22 August 1996*.

Waldron, M.E. and Kerstan, M. 2001. Age validation in horse mackerel (*Trachurus trachurus*) otoliths. ICES J. Mar. Sci. 58, 806–813.