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Analysis of jack mackerel otolith microstucture

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I N S T I T U T O D E F O M E N T O P E S Q U E R O

Chilean Jack Mackerel Age Reading Protocol

Proyecto FIP 2014-32

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Content

1. Introduction

Aguayo et. al. (1981) identified an annual periodicity formation of hyaline band of growth for the Chilean Jack mackerel (*Trachurus Murphyi)*. This is consistent with estimates of the genus *Trachurus*. However, discrimination between a real and a false annual growth is not an easy task and, in most cases, the annual growth marks do not always consist of a translucent and opaque zone, and may contain double or multiple translucent areas.

The first step in the age estimation process is to read the otolith using an incremental growth pattern appropriate for the objectives of the study; the next step is to interpret the age and provide some applications. Some inevitable interpretation criteria for the populations and/or the species must first be defined; for example, the location of the hatching check, the first increment, the transition zone, the nature of the edge, etc. The biological information available for the species should be used to define these criteria empirically. They can then be compared in order to establish an "alphabet" (identification of incremental growth patterns) and to determine the "grammatical rules" involved (the interpretation criteria based on existing knowledge) to attribute ages (**Fig. 1**). The consistency of the age estimation process then needs to be determined. This means the ability to identify the same structures consistently. The repeatability of the age estimation procedure (internal bias) must be determined and the ages calibrated (external bias).

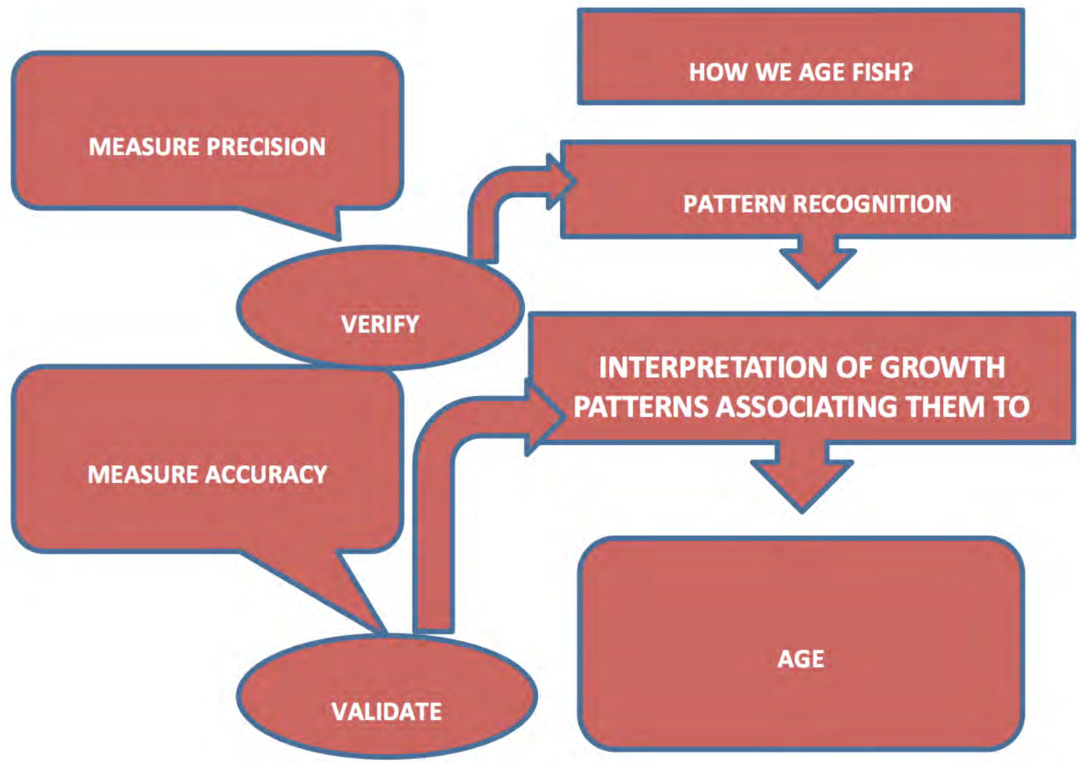


Figure 1. Steps involved in an ageing process (right panel) and quality control (left panel).

The experience of the reader (Annex 1) is very relevant to the success of limiting the bias. Even experienced readers, however, might show low levels of accuracy when starting work on a new species. Once a satisfactory level of expertise in age estimation has been reached, steps should be taken to prevent the methodology from deteriorating or changing over time, in order to maintain adequate quality of the ageing procedures. Finally, the ages must be validated, in the sense that the accuracy of the ageing has to be established. The temporal meaning of the interpreted structures must be determined in order to evaluate the closeness of the estimated age to the accurate age of the fish.

2. Otolith storage

Sagittal otoliths are cleaned with a gentle brush and distilled water, let to dry and stored dry with the due references to the biological information. The otoliths can be cleaned also in alcohol to eliminate organic tissues. As the otolith rostrum is very fragile, precautions are needed to prevent breakage. A useful method to maximize protection and reducing storage space is the use of cardboard slides with holes to place the otoliths, as applied in IFOP. Paper envelopes and vials are also widely used.

3. Otolith preparation

Fish < 45 cm FL

Whole otoliths are used for specimens less than 45 cm in fork length, both otoliths (left and right) are read without preparation (Annex 2). The otoliths are immersed in liquid glycerin on in another clarifying medium such as alcohol, in one Petri capsule with black background and are observed with a stereoscopic microscope with 10X magnification, under incident light (although a greater increase for the observation of details and identification of the type of edge is used). The use of black containers (like tops of tubes) diminishes the light diffraction. Cleaning the otolith in alcohol after reading may prevent deterioration during further storage.

Generally an image analysis system is employed coupled to the microscope and camera. Once the images are calibrated, the distances from the focus to the maximum radius of each hyaline increment are measured. The caudal otolith zone (**Fig. 2**) should be used for the measurements. Care has to be taken to draw the radius perpendicular to the growth increments.

The nature of the marginal increment should be annotated (translucent/opaque). Additionally, the degree of completion of the marginal increment could be annotated comparing with the width of the previous increment. The description could be qualitative (starting, medium, almost complete) or numerical measuring the total otolith caudal radius.

Fish > 45 cm FL

Before viewing under a low power stereomicroscope, otoliths are recoated with immersion oil or glycerin to enhance the series of alternating light and dark zones discernible in the burnt section. Initial viewing may be undertaken at low to medium magnifications (20× objectives) with illumination from a reflected cold light source to determine which of the preferred sites on the sectioned surface are the clearest for reading and to identify any visible ambiguities or secondary growth zones such as false checks.

Although most JHM ageing is undertaken using reflected light to count zones on the sectioned surface, transmitted side lighting of the otolith, particularly on the dorsal side, may be used to enhance annual zones and confirm false checks.

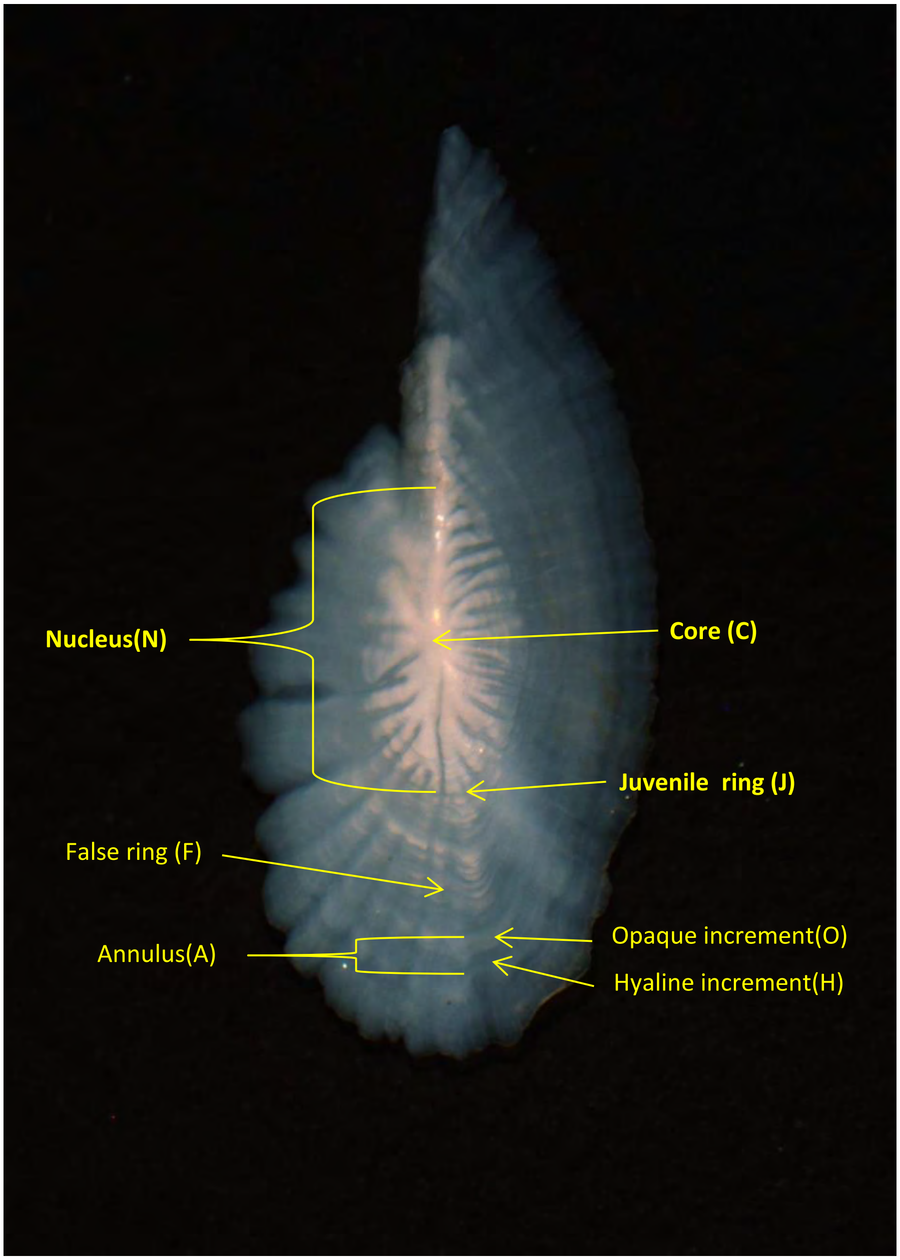


Figure 2. Sagittal otolith terminology (from IFOP).

4. Otolith interpretation

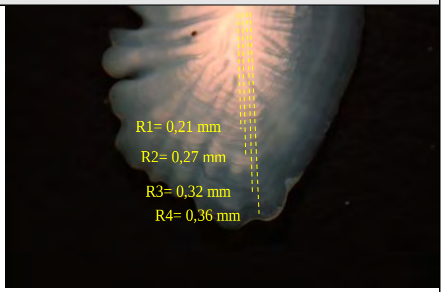
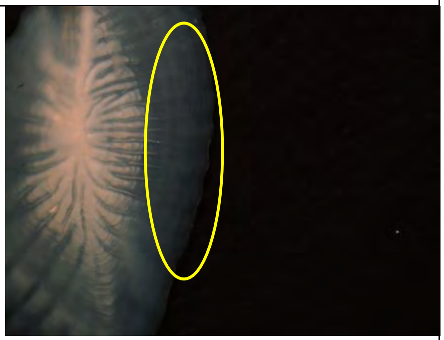
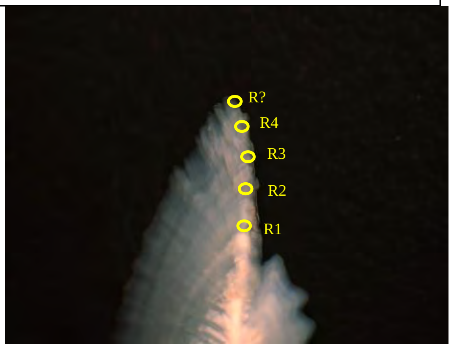
Whole otoliths

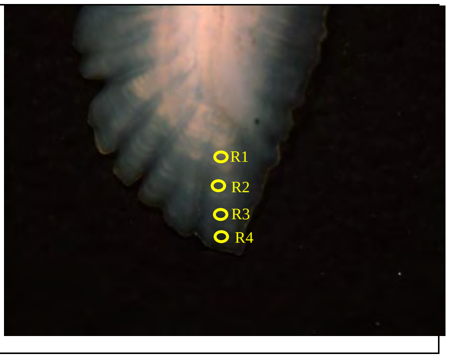
The right otolith external side is used for the interpretation. The first step is to identify the annual growth increments in the caudal area of the otolith. The continuity of the increment is followed on the ventral side of the otolith, where the nature of the marginal increment appears more clearly. The true annual increments are clearly distinct in the rostral area (Table 1). Therefore, the main interpretation criteria are: clarity and continuity of the increment along the caudal to rostral area. The increment measurements might allow to check the position of the increment and compare results between readings.

Sagitta whole otoliths show a series of false rings in the central area, which in the most transparent otoliths are numerous and seem to correspond to phenomena of short periodicity, probably related to environmental pulses as the tides. The focus is not usually evident, but can edge of the otolith. The otolith grows mainly in the caudo-dorsal area, where the lobes are more marked (Morales-Nin, 1997).

The Otoliths of horse mackerel are characterized by the presence of numerous growth rings, which are zones of rapid and slow growth that correspond to the opaque and hyaline rings of other species. A zone is often limited by an opaque ring a little thicker and a hyaline ring thin and crisp. The formation of these zones makes difficult the interpretation of the edge ring that only stands if it is complete in comparison with the previous areas.

Table 1. Steps in the whole otolith reading as applied in IFOP.

Otolith zone	Example
1-Observe Cauda and measure increments	
2-Check increment continuity in the ventral side	
3-Check the continuity of the increments in the rostrum	

4-Check the increments in the inner side of the otolith	

Transversal sections of otoliths (Fish > 45 cm FL)

The translucent zones of the otoliths appear brownish after burning. The whole left otolith is read in comparison with the sectioned right otolith. The cut surface of the otolith is covered with oil or glycerin and the otolith is placed standing with the cut surface up. The translucent increments are enumerated close to the sulcus acusticus area (**Fig. 3**). The end of the nucleus and the formation of the first increment is usually with a notch on the external side. Close examination of the sulcus edge might help identifying the increments by variations on the edge surface.

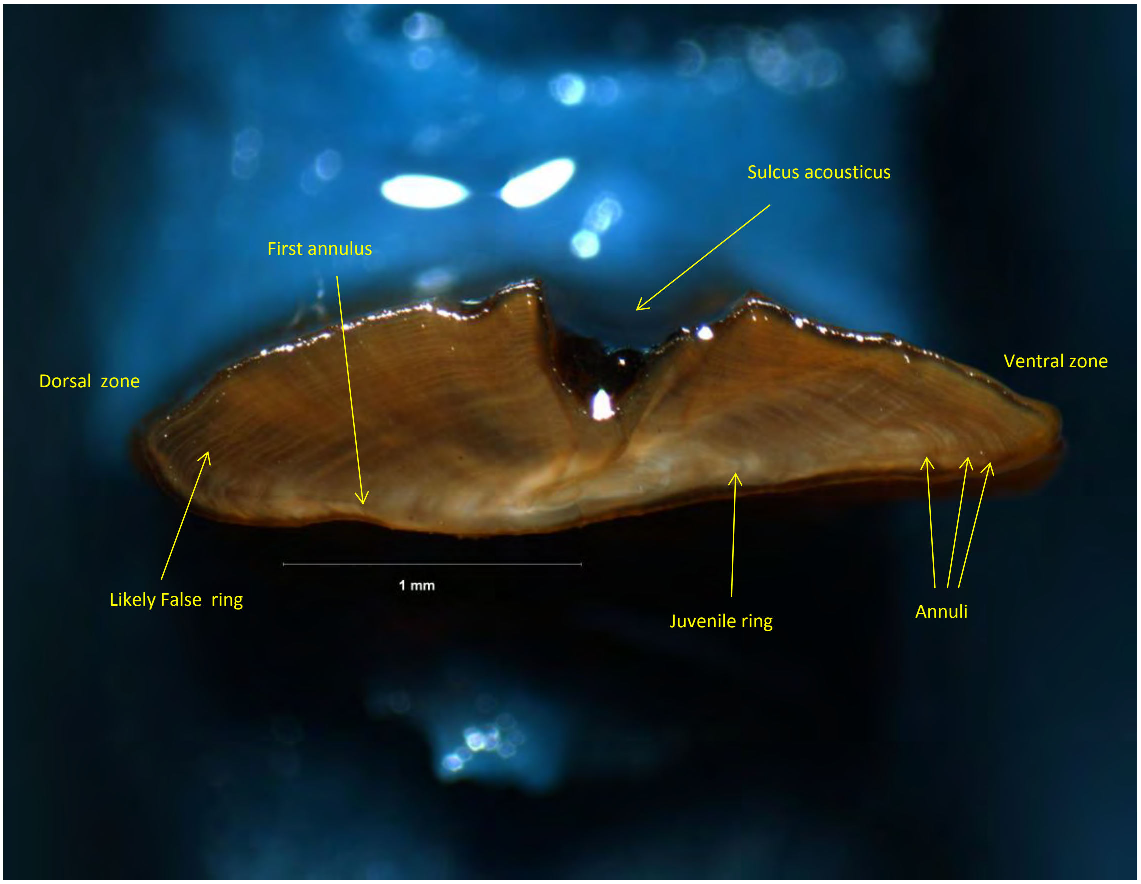


Figure 3. Transversal section of a cut and burned otolith showing the growth increments (IFOP).

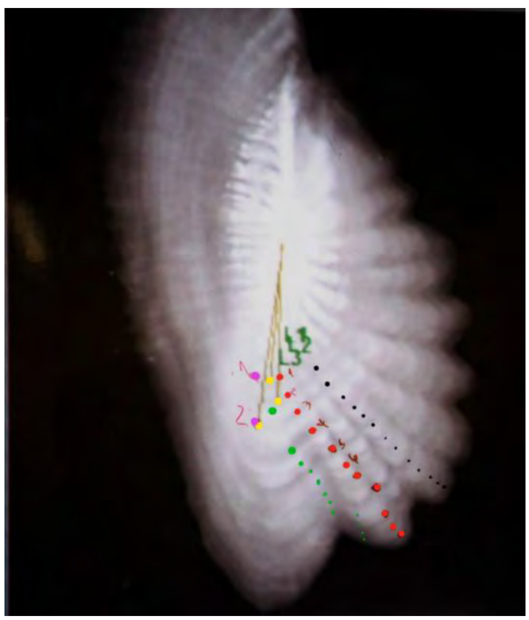
Translucent increments generally are dark and amorphous in appearance because of lower continuity of aragonite crystals. Therefore, varying the angle of incidence of the light and its intensity helps in the identification of the increments. In these cases the interpretation is preferably done in the caudo-ventral region. As in whole otoliths, the formation of zones makes difficult the interpretation of the marginal ring. To distinguish if it is complete it has to be compared to previous otolith growth areas.

In sections the first false rings appear well developed and oval in shape; however, the location of the first annual increment requires to consider its distance from the focus, the following four rings are rather fuzzy but distinguishable. Usually from the 6th ring onwards these are regularly spaced near the sulcus acusticus.

Age assignation

In large otoliths the growth zones may be confusing as pointed out by Dioses et al (2013), which showed discrepancies in increment identification and position of the increment (**Fig. 4**). This is due to the difficulty in identifying the correct increment in the growth zone. Therefore, it is recommended to use transversal sections to avoid these difficulties.

Figure 4. Otolith of a Jack mackerel *T. murphyi* of 58 cm (fork length) with alternative age readings (11 to 13 years) by different readers. Reader 1 (green dots) identifies 12 rings, reader 2 (red dots) identifies 11 rings, reader 7 (black dots) identifies 13 rings and reader 6 (purple dots) identifies the first 2 rings. The yellow dots and lines show the place and radius of the first three rings, being 2.1 mm, 2.4 mm and 2.8 mm respectively (from Serra 2011 and Goicochea et al 2013).



Annuli definition criteria

- An annulus is a bipartite structure composed by opaque and translucent zones, which ideally should not have interruptions around the entire contour of the otolith.
- The opaque zone is composed of several thin opaque-translucent increments, whilst the translucent zone usually is completed by a clear translucent ring followed by a refringent opaque increment start of the next opaque zone.
- The first annulus is considered as the limit of the first annual ring on the hyaline zone located at the radius between 1.5 mm and 2.5 mm for CJM.
- After the first annual ring, the most important criterion to identify the following areas of growth is the consistency in the distance of separation between each hyaline zone.
- The relative width of each area of otolith growth becomes progressively smaller in parallel to the decrease of fish growth, although conditions affecting the life history may alter the growth rates.
- There is a tendency to find false rings after the 1st to the 3rd annual zones, in an area that lies between 2.5 to 3.4 mm from the core to the caudal edge of the otolith.
- To define the nature of the increment in the otolith edge it is necessary to consider its width in relation to the previous increment.

False rings

In the majority of the otoliths, a clear ring is formed within a radio close to 1.5 mm. It is considered as a juvenile ring by similarity with other species. In a study currently underway using daily increments (IFOP), it has been estimated that the following macro-ring with a radius between 1.8 and 2.3 mm would have less than year periodicity. In the same study, the first ring (annulus) would be located between 1.5 to 3.0 mm distance from the focus. These distances should be confirmed in each study area.

There is a tendency to find false marks between the 2nd and 4th annual mark, in the image of the figure these are located generally between 2.1 and 3.4 mm from the focus or core towards the caudal edge of the otolith.

5. Age group and assignation to age class

The individual age group and age class are used in population and life dynamics in both of which a single age in years is required. Once all the increments on an otolith have been identified, a reader can establish the age of the fish by simply counting the number of seasonal increments on an annual basis. However, to determine the age class (or year of birthdate) it is necessary to take into account:

- Date of capture
- The peak spawning period for a given population (precise or average standard population date of birth)
- Main periods of seasonal increment formation
- Nature of the otolith edge (opaque/translucent)

In the case of CJM, due to its complex stock structure and extended reproduction period that may be from October to December, and in some cases from July to March, a standard population date of birth was accorded for 1st January regardless of hatch date (Villamor et al., 2004).

Chilean jack mackerel spawns in spring and summer (Arancibia et al., 1995), and the spawning peak appear to occur in November (Núñez et al., 2008).

The formation of the opaque increments for Chilean waters is from December to June.

Using this information and based on Panfili and Morales-Nin (2002), the working group summarized the procedure for CJM and proposed a visual example (Annex 4). This example considers the relative measure of the radius for age class attribution on age 0 and of the marginal increment development for older fish.

6. Precision

Precision is defined as the reproducibility of repeated measurements of a given structure, where these measures (readings of age) may or may not be exact (Campana 2001). The tests in the estimation of the age most commonly used are: the percentage of average error (APE) and the coefficient of variation (CV) (Beamish and Fournier 1981).

Percentage of average error (APE)

$$APE = \frac{100}{n}\sum_{j=1}^{n}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|x_{ij}-x_j\right|}{x_j}\right]$$

where,

n : number of aged fish;

R : number of times the otolith was read;

x_{ij} : i-age estimated for j-fish;

x_j : mean age determined for j-fish

The coefficient of variation and precision index (D) incorporates the standard deviation resulting in a more robust index (Chang 1982):

$$CV_j = 100\% * \frac{\sqrt{\sum_{i=1}^{R}\frac{\left(x_{ij}-x_j\right)^2}{R-1}}}{x_j}$$

where the symbols have the same meaning as the formula earlier.

There is no CV threshold value for accepting or rejecting a reading because it depends on the species and the age range. Laine et al (1991) suggested a maximum CV value of 5% as the limit for acceptance. Similarly, Campana (2001) indicates that from informal discussions among some laboratories, it has been suggested that a 5% CV would serve as reference for fish of moderate longevity and complex reading. IFOP employs a value of the APE of 10% as maximum permissible error. This percentage may occur without the existence of trends, which are evaluated with the linear relationship of reading between two readers, which should be similar to the bisector (slope equal to 1).

7. Validation

Once an ageing structure has been identified, their temporal significance has to be ascertained. Thus, their periodicity has to be validated. There are several methods for age validation (Campana 2001) that have different resolution, limits and advantages. Therefore, we recommend validating the age estimates using several validation methods when possible because there is not a single one that covers all the age range present in the fishery. CJM complex zonation requires several approaches for a complete validation.

8. Quality control

Age determination using otoliths implies a repetitive process of interpretation of growth zones that implies visual processes and biological knowledge. Moreover, individual fish variability, environmental changes and biological processes may alter the appearance of the zones. Faced with the multiple source of bias, a quality control must be performed.

Age determination is subject to error from two major sources: error inherent in the structure itself, and error in the process of interpreting its incremental structure (Campana 2001). The first type of error can be estimated but not controlled; the second type of error can be both estimated and controlled (Morrison et al. 2005). Errors in the estimation process can affect either accuracy (closeness to the true value) or precision (closeness of repeated estimates) (Kalish et al. 1995). The prevalence and impact of inaccurate age determinations on the accuracy of population dynamics studies cannot be overstated (Campana 2001), and the issue of accuracy, the purpose of age validation, has received deserved attention since the important paper by Beamish and McFarlane (1983).

Quality control (QC) is normally equated with age validation, which is often difficult and expensive to undertake. However, validation is only one of the three components of QC, and in some cases, is the smallest source of error. All ageing studies which involve more than one set of ages, whether at the daily or yearly level, should incorporate a complete QC program containing the following:

- age validation - demonstration that the age based on counts of periodic growth increments is, on average, equal to the true age of the fish.
- tests for bias and long-term drift - demonstration that the age reader interprets the growth increments in the same way (on average) as other age readers and at other times.
- measures of precision - measures of repeatability among age readers or within the same age reader on different occasions.

Validated or not, different age readers can easily interpret a given otolith in different ways. If the difference is consistent - that is, one reader is higher or lower than the other for one or more age groups, at least on average - there is a bias. These biases between readers have been detected on the CJM otolith interchange exercise performed as well as in the previous Ageing Workshop (Annex 5).

A bias may also occur within a reader over a period of time, such that a given age reader interprets an otolith differently now than was the case a few years ago. Standard measures of precision such as CV, APE and percent agreement do not detect such a bias, particularly if it occurs only in old fish. Nor can replicate readings of a sample taken from the current year detect long-term drift. However, an age bias plot is well suited to detecting bias, and should be a standard component of any ageing program. Measures of precision are meaningless if bias is present. However, if bias is absent, the coefficient of variation (CV) and average percent error (APE) are both useful measures.

For a QC the following steps should be considered:

- Elaborate detailed protocols for each step of the ageing process.
- Determine precision and accuracy of the ages regularly.
- Determine training programs both for the new readers and for the experienced ones, to ensure that they maintain the same ageing criterion along time.
- Build up and use reference collections, both of otoliths and their images. Ideally, these collections must be of known age fish. In absence of them, otoliths with high repeatability and with ages validated could be used.

- Maintain regular workshops, to ensure that the age readers from different institutions and assessing the same resource, have homogeneous criteria.
- Perform exercises to support the age estimates such as:
  - Compare the obtained growth curves and growth parameters.
  - Inspect scatter plots of age versus otolith weight or fish length.

It has to be considered that ageing is a skill and thus, it can be learned. The elements of the process can be taught, learned, and practiced. The level of skill increases with practice and experience, and will decline with time without practice. Some practitioners are naturally far more skilled than others. All practitioners vary in the skill they display at different times (Morrison et al 2005).

ACKNOWLEDGEMENTS

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Annex 1. Characteristics of the participants in the otolith reading interchange and in the Workshop.

Professional	Reading experience	Expertise	Otoltih reading	Workshop	Afiliation
Christian Valero	Medium (3 yr)	Routine age determination (more than 2400 otoliths read/year)	YES	YES	IFOP, Chile
Lizandro Muñoz	High (15 yr)	Routine age determination (more than1800 otoliths read/year)	YES	YES	
Miguel Araya	High		NO	YES	Universidad UNP
Teobaldo Dioses	Medium	Research project	NO	NO	Instituto del Mar del Perú (IMARPE), Peru
Carlos Goicochea	Low	Research project, microstructure	NO	NO	
Natalia González	Low	Expert in *Scomber japonicus*	YES	YES	Instituto Nacional de Pesca (INP), Ecuador
Kordian Trella	Participation in the 2011 Workshop	Biology	NO	NO	Morski Instytut Rybacki (MIR) - Państwowy Instytut Badawczy, Poland

Professional	Reading experience	Expertise	Otoltih reading	Workshop	Afiliation
Jacobus De Klerk	High (5 yr)	Routine age determination (more than 1000 otoliths read/year)	YES	NO	Corten Marine Research (CMR), Netherlands
Nikolay Timoshenko	Medium	Occasional studies Definition of ageing criteria	YES	NO	Rusia
Gang Li	Medium	Occasional studies	NO	NO	Changhai Ocean University, China
Guoping Zhou	?		NO	NO	Changhai Ocean University, China

Other participants in the Workshop: Ignacio Paya, IFOP, Mario Acevedo, SUBPESCA.

Annex 2 Terminology used in the protocol, from IFOP with modifications.

Name	Symbol	Sinonim	Definition
Focus	F	Primordium	The otolith center, laid down during the first life phases. Sometimes not visible due to thickening of the otolith with growth.
Nucleus	N		Central portion of the otolith generally more refringent than the rest, surrounded by the first translucent zone. It may contain several translucent increments caused by life history transitions.
Translucent increment	H	Hialine Increment/ring	Translucent growth increment, frequently composed by a zone of thin alternate O/H rings, laid down during fast growth periods.
Opaque increment	O	Opaque ring	Opaque growth increment, frequently composed by a zone of O/H thin rings, laid down during slow growth periods.
Annual growth zone	Arabic numbers	Annual ring Annulus	An opaque area adjacent to a hyaline area, interpreted as a band of annual growth. In the zone most distal (flow) of otolith these zones are close and with higher resolution (is identified as a single ring more easily)
False ring	F	Check	Growth increment that can not be identified as an annual growth zone. These are not visible in all of the otolith, are thinner and had lower resolution.
Age class	Roman numbers		Age determined from the otolith interpretation, date of capture, birth date and time of annual increment formation. Corresponds to the year of birth.

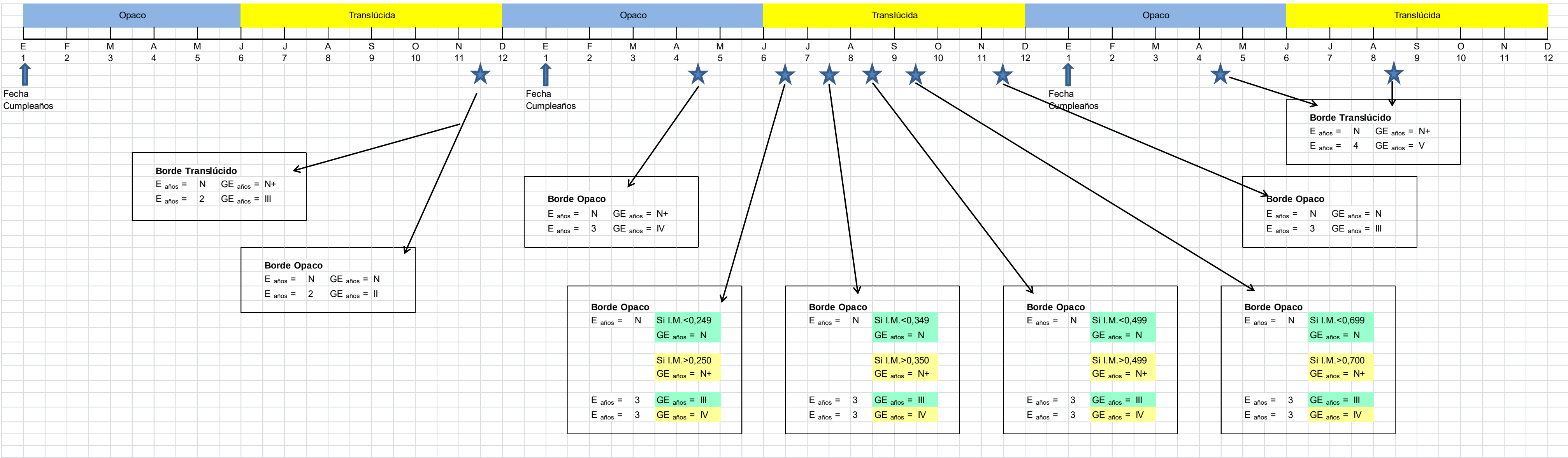
Annex 3. Laboratory procedures

Institution	Otolith storage	Otolith preparation	Otolith interpretation	Validation/Reference collections	Ageing criteria
IFOP, Chile	Chilean jack mackerel otoliths are stored in sheets of 24 pairs of otoliths (clean and dry). We use identification data such as time and geographical position. After analyzed, the otoliths are returned to their sheets and stored.	For specimens smaller than 45 cm of fork length (LH), whole otoliths (left and right) they are used. For specimens larger than 45 cm(FL), the right otolith is used, cross section, polished and roasted according to the method of Moller-Christensen (1964).	For whole otoliths, the pair of otoliths is deposited in a Petri dish with black background and baseline and observed with a stereoscopic microscope at 10X magnification under incident light. The maximum radius of each hyaline mark is registered. The type of border (Hyaline / Opaque) is also identified and estimated marginal increment. For cross-sectioned otoliths, a thin layer of liquid petroleum jelly on the polished surface of the sectioned otolith and is observed with a stereoscopic microscope with 20X magnification under incident light. Hyaline marks near the sulcus acoustic counted.	For the first ring: Validation by the method of daily micro increments. (FIP 2014-32 results) For the second and third ring: Method marginal increases. (FIP 2014-32 results) Reference collection (150 images in TIFF format)	We consider as a real annual growth ring a opaque band (Fast grow) followed by a traslucent band (Low grow), which should not present disruptions around the contour of the otolith. The limit of annual first ring (hyaline band) is among 1.5 mm and 2.5 mm for the following annual rings, the most important to identify growth rings following criterion is consistency in the separation distance between each hyaline brand.
Instituto Nacional de Pesca (INP), Ecuador	Clean and dry sagitta otoliths in envelopes	Otolith is placed in black containers with immersion oil, the hyaline rings are observed as dark bands.	For reading reflected light is used under a stereomicroscope Preferred reading area: near the acoustic groove		The most appropriate areas for determine the number of rings are the Rostrum and caudal edge. The union of the opaque and traslucid area is considered a year increment The distance to the core of the

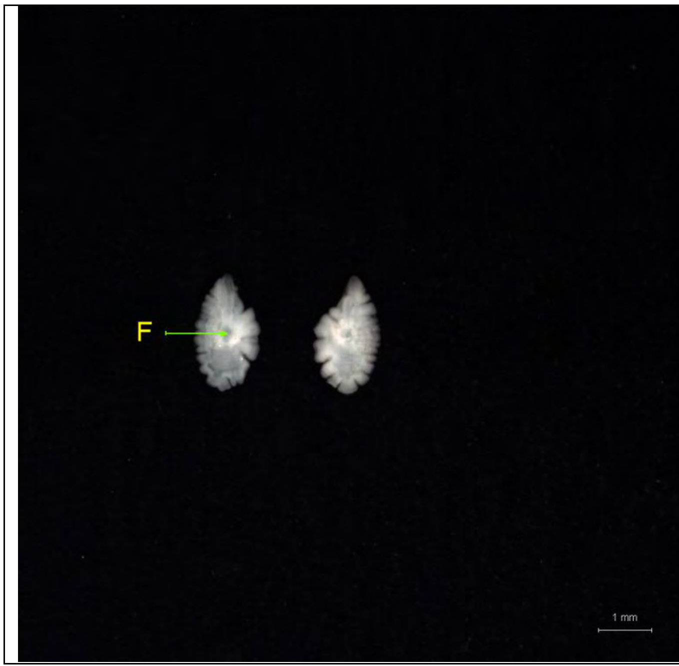
Institution	Otolith storage	Otolith preparation	Otolith interpretation	Validation/Reference collections	Ageing criteria
					first ring is larger than this subsequent rings and decreases close to the otolith edge. The true rings usually must be continued with the contour of the otolith.
Corten Marine Research (CMR), The Netherlands	Clean and dry sagitta otoliths in sheets (of 25 pairs of otoliths), when we fish we make 1 sheet each day, fish are taken at random and are also used for the biological sample.	Only whole otoliths are used.	Immersed in water in a black container using incident light. Most studied is the inner caudal part of both otoliths and I try to get confirmation of the result in reading the rostrum (and sometimes other parts of the otolith)	I compare results with an Age Length Key obtained by my length measurements while we had several years without recruitment and could clearly follow the yearly growth of a year class.	Continuity of the rings around the otolith; regular spacing and if possible confirmation of the rings in the rostrum.
AtlantNIRO, Russia	Clean and dry sagitta otoliths in envelopes	Whole otolith. Broken when appropriate	Immersed in water in a black container using incident light Preferred reading area: whole otoliths caudal and anterior sections Radius distances measured where necessary	Increment analysis	Relative spacing of the rings and continuity around the otolith
Shanghai Ocean University, China	Clean and dry sagitta otoliths in envelopes	Transversal thin sections cut for young and old fish	Smeared glycerin with black background, using incident light and read the image taken by CCD	Marginal increment analysis for adults	Annuli:Thin clear translucent increments

Institution	Otolith storage	Otolith preparation	Otolith interpretation	Validation/Reference collections	Ageing criteria

Annex 4. Age class assignation for CJM. 1st January birthdate.



Annex 5. Images from the otolith interchange read jointly in the Workshop.



Otolith Reference	8
Exploitation area	Iquique
Capture	January 11, 2011
Fish Length (cm)	8
Age (years)	0
Age Class (years)	0

Comments:

The focus (F) appears clearly. Note the lobulations in the otolith periphery typical of young fishes. Otolith radius 1.1 mm.

Otolith Reference	23
Exploitation area	Iquique
Capture	March 22, 2011
Fork fish Length (cm)	16
Age (years)	0
Age Class (years)	I
Comments: Several false rings. Incomplete translucent zone in the edge. Otolith shape starts to change along the ventro-dorsal side.	

Otolith Reference	34
Exploitation area	Iquique
Capture	June 12, 2012
Fork length (cm)	20
Age (years)	2
Age Class (years)	III
Comments: Opaque edge in formation (Marginal increment: 0.382).	

Otolith Reference	9
Exploitation area	Iquique
Capture	April 24' 2012
Fork fish Length (cm)	27
Age (years)	3
Age Class (years)	IV
Comments: Example of increments composed of growth zones. Opaque edge (0.650).	

Otolith Reference	1
Exploitation area	Arica
Capture	May 4, 2012
Fork fish Length (cm)	27
Age (years)	4
Age Class (years)	V

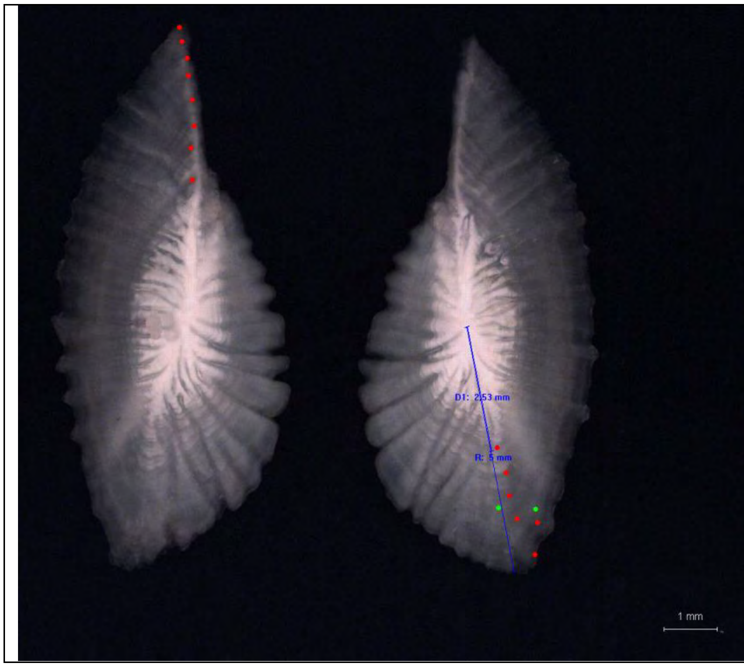
Comments:

Decreasing increment width according to the growth pattern.
Opaque edge (0.16)
First green dot consensus reading, red dots agreement reading with Russian reader.

Otolith Reference	112
Exploitation area	Peru
Capture	
Fork fish Length (cm)	29
Age (years)	4 o 5?
Age Class (years)	

<u>Comments:</u>
Location of the first and second increments uncertain (green dots).
Opaque edge (0.902)

Otolith Reference	89
Exploitation area	Ecuador
Capture	February 1,2013
Fork fish Length (cm)	39
Age (years)	6
Age Class (years)	VI
<u>Comments:</u> First increment location determined using the standard radius. Translucent increment in the edge.	

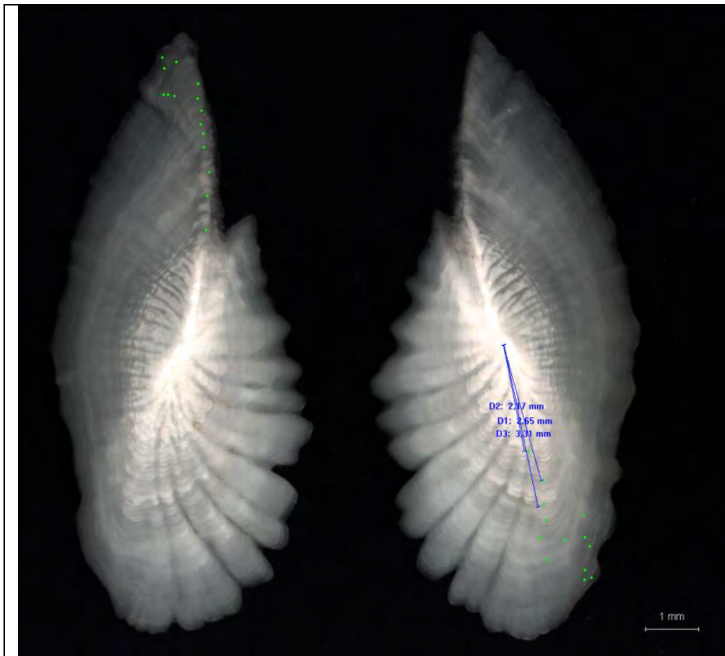


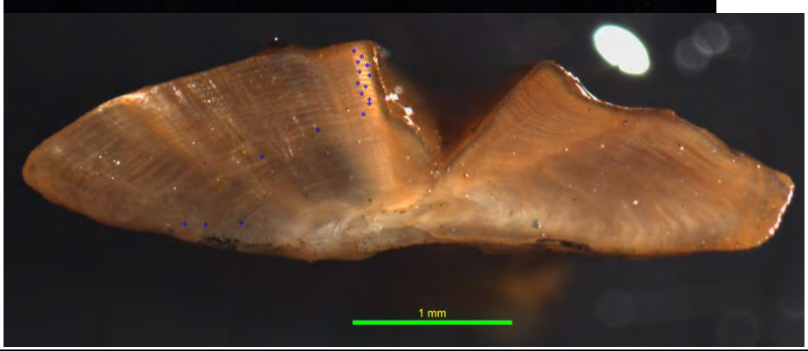
Otolith Reference	147
Exploitation area	Peru
Capture	
Fork fish Length (cm)	40
Age (years)	8
Age Class (years)	

<u>Comments:</u>
Clear pattern in the rostrum. Red dots Russian reader, green group reading. Left otolith group reading.

Otolith Reference	105
Exploitation area	Peru
Capture	
Fork fish Length (cm)	30
Age (years)	5
Age Class (years)	

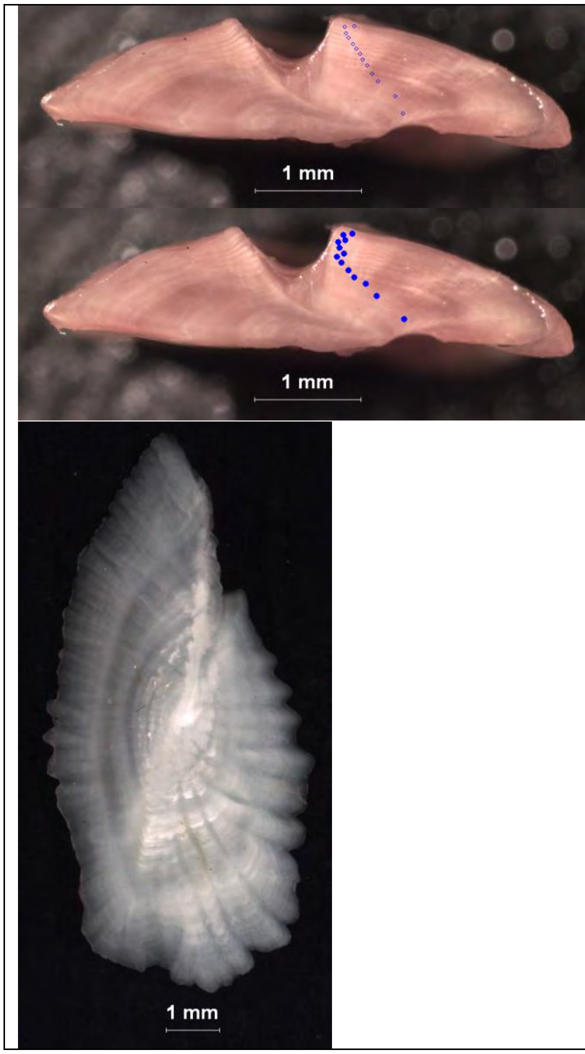
Comments:
In caudal area 4, clear 5 increments in rostrum. Opaque edge (1)





Otolith Reference	2
Exploitation area	Coronel
Capture	April 9, 2012
Fork fish Length (cm)	52
Age (years)	18
Age Class (years)	XIX

<u>Comments:</u>
Narrow increments close to the edge. The image below corresponds to transversal section (20x magnification). Blue dots on section show the correct reading



Otolith Reference	Not from otolith interchange
Exploitation area	Talcahuano
Capture	September 25, 2006
Fork fish Length (cm)	
Age (years)	13
Age Class (years)	XIV
Comments: Example of a whole and transversal otolith section (both sides of the section) showing the increased clarity of adult increments in the sections.	