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Workshop on micro increment daily growth in European Anchovy and Sardine (WKMIAS)

21–25 October 2013

Mazara del Vallo, Sicily

ICES
International Council for the Exploration of the Sea
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International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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Contents

Executive summary

The Workshop on Micro increment daily growth in European Anchovy and Sardine (WKMIAS) met for the first time from 21-25th October 2013 in Mazara del Vallo, Sicily (CNR-IAMC). The meeting was chaired by Gualtiero Basilone (CNR-IAMC, Italy), Begoña Villamor (IEO, Spain) and Mario La Mesa (CNR-ISMAR, Italy). Six nations were represented by 22 participants.

WKMIAS was proposed by the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS) 2012. Many activities of this group are closely linked to the activities of the Data Collection framework (DCF). Although under the ICES have been made multiple age reading workshops at annual scale, this was the first time that a workshop was dedicated exclusively to age reading at daily scale. The objectives of the workshop were to define and standardize methods, reading criteria and protocols of anchovy and sardine daily growth in different developmental stages (larvae and juveniles), and validate the first annual ring of these species to improve annual age estimates. The report summarizes the work in relation to each of the ToRs.

Given the terms of reference and objectives, the daily growth of these species is dealt with the following geographical areas/stocks/ecosystems: Bay of Biscay, Atlantic Iberian Peninsula (sardine only), Western Mediterranean, Strait of Sicily, Adriatic Sea and North Aegean Sea.

Before the workshop, a questionnaire was performed in which each laboratory should provide information on their method of preparation and interpretation of the anchovy and sardine otolith microstructure. The result of them was reviewed and summarized, reaching a common protocol during the workshop regarding the methods and techniques of preparation of the otoliths. However, there was not agreement in relation to daily increment interpretation criteria.

The workshop also was preceded by an otolith image exchange, which was undertaken using EARF in the months prior to the workshop. The exchanged otolith collection included 81 images (41 for anchovy and 40 for sardine) distributed in 10 sets from different anchovy and sardine distribution areas. In the case of sardine, also a small otoliths collection (5) of known age (obtained from Aquaculture) was used. The exchange proved the existence of differences between readers and areas of both species, with a precision ranging from 9.0 to 34.9% CV for anchovy, and from 9.4 to 18.0% CV for sardine. The comparison with the actual age of sardine (known from marine culture) showed that sardine readers are generally in good agreement; nevertheless all readers underestimated the older fish.

During the workshop a reading exercise on live images of anchovy and sardine otolith thin section was carried out. The main aim of such exercise was to increase the agreement among readers and to highlight differences due to the interpretation criteria adopted, or due to the differences in the growth pattern among areas. There were two criteria for interpreting the anchovy and sardine daily Micro increments according to double bands (called GBR) or individual bands (IMR) form. The application of these criteria was recurrently discussed during the workshop, not being possible reach a unanimous agreement on standardization. So it was agreed to use the GBR in all areas, except for the Strait of Sicily and Adriatic Sea.

Another of the main issues of the workshop was to identify the position of the first annual ring (annulus) in the otoliths of these species, since it is one of the main sources

of error that affect the precision of age. Based on different daily growth studies presented at this workshop, the position of the first annual ring is validated on anchovy in the Bay of Biscay and the position of the first ring false or check is corroborated in the sardine of North Adriatic Sea, providing a series of recommendations to the annual ageing readers of these species.

A reference collection of otolith images was also provided for larvae and juveniles of anchovy and sardine from each area. Finally, a literature review of recent research related daily growth of these species was performed and a new Workshop on Micro increment daily growth in European Anchovy and Sardine was proposed for 2017.

1 Introduction

A number of studies as much as age readers meetings and workgroups highlighted the importance to carry out the validation of first annual ring in calcified structures of bony fish. Despite such importance, the WKMIAS represents the first workshop on this topic and several activities were carried out for the first time.

In the case of Small pelagics, the standardization of methodologies and the validation of the first annulus assume much importance because of their short lifespan. In species with 3 to 4 years of maximum age the misinterpretation of one check (false annual ring) as an annulus would determine a high variability of the age stock structure results and thus great variability of the stock assessment and management studies. Such a big uncertainty would be greatly reduced if the assignment methods could be previously validated by daily increment studies. Behind the validation of the first annulus, the standardization of thin section preparation techniques and mainly the standardization of age assignment and ring interpretation criteria affect heavily the comparability of results among scientists/areas. To overcome such a problem, several months before the workshop it was started a programme of exchange of material and age assignment data to compare results among readers.

Furthermore, during the workshop at the sclerochronology lab of the "Istituto Ambiente Marino Costiero-Consiglio Nazionale delle ricerche" (IAMC-CNR), each lab/Institution carried out a reading exercise on live images of the otolith thin section. Each participant brings from his/her lab larvae and juvenile otoliths of anchovy and sardine ready for analyse by mean of a microscope and an image processing system. The main aim of such exercise was to increase the agreement among readers and to highlight differences due to the interpretation criteria adopted, or due to the differences in the growth pattern among areas.

A particular attention was devoted also to produce standard protocols for the materials and techniques used for the preparation and analysis of otolith thin sections. Such protocols take also into account differences in the otolith structure among areas due to different rings/check patterns, suggesting different solutions.

A reference collection of otolith images was also provided for larvae and juveniles of anchovy and sardine from each area.

Opening of the meeting

The opening of the meeting was held on Monday 21st October 2013 at 9.00 a.m. by the Chair Gualtiero Basilone (IAMC-CNR, Mazara del Vallo).

Professor Salvo Mazzola, Director of the "Istituto Ambiente Marino Costiero" (IAMC-CNR) opened officially the WKMIAS work, welcoming to the meeting all the participants and wishing them a productive and stimulating journey in Sicily and especially in the new Institute which host the working group along the week.

The Chairmen highlighted the important role covered by ICES, EU and Italian Ministry for agricultural and fishery policies (MIPAAF) in supporting the workshop. He also thanks for coming the two co-chairs: Begoña Villamor (IEO- Santander) and Mario La Mesa (ISMAR-CNR, Ancona).

After this introduction, each participant presented himself to the others (the complete list of participants is presented in Annex 1).

Each WG member presented its Working Document to the entire group (full presentations are available on the ICES Share point web page: https://groupnet.ices.dk/WKMIAS2013/default.aspx

Adoption of the Agenda

The draft agenda was presented to participants who made some adjustment based on the big amount of work to carry out within the time duration of the meeting, and also taking into account time for standardization exercise among readers which met for the very first time on such topic. The final agenda was approved as in Annex 2.

Terms of Reference

a) Review literature and consider recent research to define daily increment patterns in anchovy and sardine;
b) Standardize Material and methods for preparation of otolith thin sections;
c) Define and standardize the daily age reading criteria among areas;
d) Validate the first *annulus* in young of the year anchovy and sardine;
e) Determine growth-rate pattern of juvenile anchovy in different areas/environments;
f) Estimate precision and accuracy of age estimates by micro-increment counts;
g) Create a reference collection of otoliths and start the development of a database of otolith images.

Data from MS institutes (questionnaire sent to institutes before the meeting)

The WKMIAS represents the first attempt to address micro-increments growth to validate the first annulus formation. To this aim, a relevant amount of information regarding techniques and methodologies adopted to study daily increment formation by areas and Institutes was totally missing before the workshop. To overcome such a gap and to allow the planning of the workgroup activities before and during the WKMIAS, it was sent a questionnaire to each participant. The questionnaire is available in this report as annex 8 while results are resumed in section 4 of present report.

2 Ecosystem and biology considerations for anchovy and sardine populations

2.1 Anchovy

The European anchovy (*Engraulis encrasicolus* L. 1758) is widely distributed along the northeastern and Central Atlantic (coasts of Europe, about South Bergen, Norway, but not in the Baltic and it is rare in the North Sea); in the whole Mediterranean and Black and Azov seas, southward along the coast of West Africa to South Africa.

In European Atlantic waters, anchovy stock in Bay of Biscay is considered to be isolated from a small population in the English Channel and from the population in the area IXa. No subpopulations have been defined, although morphometrics and meristic studies suggest some heterogeneity at least in morphotipes (Prouzet and Metuzals, 1994; Junquera and Perez-Gandaras, 1993). Some genetic heterogeneity based on proteins allocime loci have been found between the Garonne spawning regions and southern regions in the Bay of Biscay (Adour and Cantabrian shores; Sanz *et al.*, 2008). Nevertheless, the evident inter connection of fisheries and rather homogenous recruitment pulses occurring in the Bay of Biscay lead ICES to consider that the anchovy in this area should be dealt as a single stock for assessment and management (ICES 2007).

In the Mediterranean Sea, no geographical pattern has evidenced by morphometric and shape studies of body and otoliths (see Pla *et al.*, 1996), but recent studies based on mitochondrial analysis revealed a complex mosaic of 4 genetically differentiated populations in the Mediterranean Sea: northwestern Mediterranean (including Cadaqués, Gulf of Lyon, Elba and Sicily), southern Adriatic, northern Adriatic and Aegean Sea (Viñas *et al.*, 2013).

Anchovy has an indeterminate fecundity: oocytes continuously differentiate in the ovary during the spawning season. Anchovy is sexually mature at age-1 in its first spring. Spawning takes place from spring to autumn. In the Bay of Biscay the spawning season takes place mainly between April and July, whereas in the Mediterranean Sea, the spawning period is more prolonged, even from March to November (ICES, 2010).

Anchovy in the Bay of Biscay may grow to >20 cm and live span rarely goes beyond three years. Anchovy grows very fast during the first year, doubling its weight from first to second year of life, reaching 6-7 cm in September and later, from October to November reached successively, 8 to 12 cm. The following spring, measured about 13.5 cm. At the end of his life, generally when anchovy is 3 years old, his average length is about 18 cm. Half-life is around 2-3 years, not exceeding 4 years (Hernández *et al.*, 2009).

Aldanondo *et al.*, (2011) reported the growth of young-of-the-year European anchovy (*Engraulis encrasicolus,* L.) in the Bay of Biscay through analysis of the otolith microstructure. Anchovy larvae and juveniles were sampled during their growth season over a three year period (2004, 2005 and 2006). Anchovy ranged between 3.1 and 146.8 mm (standard length) for individuals of between 1 and 108 days old. A logistic curve was fitted to the length-at-age data. At the end of the first year of growth, length was 168.3 mm, with a maximum growth rate of 1.85 mm/day for 73 days after hatching. Moreover, a Gompertz growth model was applied to estimate otolith growth parameters. At the end of the first year the otolith radius was 1759 μm, with a maximum growth increment of 22.19 μm/day observed 56 days after hatching. Standard length and otolith radius were closely related; however, this relationship showed a characteristic change from exponential in the larval stage to linear in the juvenile stage.

In the Mediterranean Sea, data from Adriatic Sea show that anchovy grow up to a maximum of approximately 20 cm, which corresponds to a lifespan of 6 years. The fastest growth in length and weight occurs in the first 3 years, asymptotic length being attained in the fourth year (3+; Sinovčić, 2000). Furthermore, growth was faster in males although they reached lower asymptotic lengths.

La Mesa *et al.*, 2009 reported the growth rates of juvenile anchovy (*Engraulis encrasicolus*) estimated from monthly samples collected in the coastal waters off Ortona (central Adriatic Sea). Otolith microstructure analysis was conducted on specimens ranging from 10 to 60 mm TL. Age estimates ranged from 16 to 96 days, hatch date distribution, back-calculated from the date of capture, was spread over a long period (April-February), with three main cohorts hatched in May, August and November. The instantaneous growth rate, calculated for the whole fish sample, was 0.68 mm/day. Nevertheless, the growth rate estimated for each of the main cohorts showed a seasonal trend, being higher in May and decreasing in August and November. The allometric relationship between otolith and fish size changed markedly during the transition from larval to juvenile stages, with a slight decrease of otolith growth rate after metamorphosis.

2.2 Sardine

Sardine (*Sardina pilchardus*) is distributed in the Northeast Atlantic from the southern Celtic Sea and North Sea to Mauritania and Senegal, and also across the western and northern Mediterranean Sea (Parrish *et al.*, 1989).

From the results of the SARDYN Project and other recent studies (e.g. Laurent *et al.*, 2007; Gonzalez and Zardoya, 2007), there is a single genetic stock of sardine in the north-Atlantic, which spans the continental waters from the Agadir area in north Morocco to the North Sea. Other evidences, such as the distribution of eggs and adult fish, body morphology and growth suggest mixing between sardine from ICES Sub-Area VIIIa and b and the actual assessed stock area. The extent of such mixing is still uncertain and the influence on the whole Iberian stock is unknown (Riveiro *et al.*, 2012). In the Mediterranean Sea, 8 stocks are considered, the largest ones located off the northern Spanish waters and the Gulf of Lions (FAO, 2009), showing great genetic differences with Atlantic populations (Kasapidis *et al.*, 2012).

Sardine is a batch spawner with indeterminate fecundity (e.g. Ganias *et al.*, 2007). In European Atlantic waters, the main spawning period is between October and June (peak December, March), with a latitudinal gradient in the duration and peak of the season, i.e. longer duration and earlier peak in the south (e.g. Coombs *et al.*, 2006; Stratoudakis *et al.*, 2007). Off the Portuguese waters, the duration of the spawning season is 5-7 months and the peak varies between December and February (Nunes *et al.*, 2011). From western Iberia to northern France, peak spawning shifts progressively to late spring months and in the English Channel there is a clear double peak, with the main periods of spawning occurring in spring/summer and again in autumn (Southward *et al.*, 1988; Solá *et al.*, 1992; Stratoudakis *et al.*, 2007).

Growth is strongly seasonal, taking place mostly outside the spawning period. Growth in length is greater in late spring and summer. Body condition and fat content of sardines peak in early autumn (when spawning resumes), and the lowest values are observed in late winter/early spring with the cessation of spawning (Bandarra *et al.*, 1997; Nunes *et al.*, 2011). Growth declines from north to south resulting in clinal variation in maximum length and length-at-age (Andreu and Plaza, 1962; Silva *et al.*, 2008). Geographical variations are generally smooth but result in substantial differences when distant areas are compared; for example, at young ages, sardines from north France are larger than those from north Portugal by ca. 2 cm, and the latter are larger than those from the Gulf of Cadiz by ca. 1 cm. Male and female sardines have diverging growth trajectories, however the difference in length is small up to age 6 (Silva *et al.*, 2008).

3 Review literature and consider recent research to define daily increment patterns in anchovy and sardine

3.1 Introduction

WKMIAS represents the first daily growth workshop in the ICES framework.

Between 2007 and 2010 the European project SARDONE (Improving assessment and management of small pelagic species in the Mediterranean) was carried out, which so far is the only project funded for daily growth studies on these species. In the framework of this project, several exchanges and workshops for daily growth in both larvae and juvenile were carried out, in order to standardize methods for sample preparations and reading criteria and some reports and publications were produced. A common ageing protocol was established (Morales-Nin *et al.*, 2010).

During the WKMIAS workshop, extensive literature on fish daily growth for many species and areas, regarding preparation of the samples, reading criteria, growth pattern, etc., has been reviewed. Table 5.5.1 shows the revision of the existing literature, made for all the participants during the workshop, in an attempt to collect also grey literature from the study area, with limited distribution (as project, workshops and working group reports, etc.) that could be available in the Age Forum Web (https://groupnet.ices.dk/AgeForum/WKMIAS/Forms/AllItems.aspx).

Most of the works produced were related with methodology for sample preparation and reading criteria, and there is much more literature for anchovy than for sardine.

Daily growth studies for these species in European waters started in the mid-80s, with a significant increase since the decade of 2000. For sardine, most of the publications are related with environmental factors affecting growth and recruitment, and there is little work on validation. In the case of anchovy, as well as studies related to the impact of environmental factors on growth and survival, during WKMIAS workshop we have compiled abundant information about validation of daily growth in culture and in the natural environment.

3.1 Table with relevant literature

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
Aldanondo *et al.* (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	Anchovy		Validation	Daily increment deposition in the otoliths of anchovy larvae
Aldanondo N (2010) Young of the year European anchovy in the Bay of Biscay. Study of recruitment determining processes based on otolith microstructure analysis. PhD Thesis. University of the Basque Country.	Anchovy	Bay of Biscay	Anchovy Ecology	Anchovy recruitment study based on otolith microstructure analysis

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
Aldanondo *et al.* (2010) Growth and movement patterns of early juvenile European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay based on otolith microstructure and chemistry Fish. Oceanogr., 19: 196–208	Anchovy	Bay of Biscay	Anchovy Ecology	Anchovy juvenile growth and movement patterns based on otolith microstructure and chemical analysis
Aldanondo *et al.* (2011) Growth of young-of-the-year European anchovy (*Engraulis encrasicolus*, L.) in the Bay of Biscay. Sci. Mar., 75(2): 227–235	Anchovy	Bay of Biscay	Age and Growth	Growth of young-of-the-year anchovy
Alemany F, Alvarez F (1994) Formation of initial daily increments in sagittal otoliths of reared and wild Sardina pilchardus yolk-sac larvae. Mar. Biol., 121: 35-39.	Sardine	NW Spain (Atlantic Iberian)	Sample preparation. Determination of the first increment in sardine	Hatch check at 5-7 μm
Alemany *et al.* (2006) Postflexion larvae and juvenile daily growth patterns of the Alborán sea sardine (Sardina pilchardus Walb.): influence of wind. Sci. Mar., 70(2): 93-104	Sardine	Alborán Sea	Relation of survival and growth (larval and juveniles) with the wind	Daily growth pattern in juveniles of sardine Evolution of increments widths during the first months and its relation with the date of birth
Allain G *et al.* (2003) The selection process from larval to juvenile stages of anchovy (*Engraulis encrasicolus*) in the Bay of Biscay investigated by Lagrangian simulations and comparative otolith growth. Fish. Oceanogr. 12:4/5, 407–418	Anchovy	Bay of Biscay		
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.	Sardine	NW Spain (Atlantic Iberian)	Growth and recruitment	
Alvarez F, 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.	Sardine	NW Spain (Atlantic Iberian)	Birthdate and recruitment	

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
Alvarez F, Butler JL (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.	Sardine	NW Spain (Atlantic Iberian)	Birthdate and environmental factors	
Anon. (1993) Report of the Clupeoids Otolith Microstructure Workshop. Inf. Tec. Inst. Esp. Oceanogr., 145	Anchovy	European waters	Daily age reading criteria	
Arneri *et al*. (1998) Growth of juvenile anchovy, Engraulis encrasicolus, in Central Adriatic Sea. In Second International Symposium on Otolith Research and Application, Book of Abstracts. Bergen, 195.	Anchovy	Adriatic Sea		
Belchier *et al.* (2004) Recruitment studies: manual on precision and accuracy of tools. ICES Techniques in Marine Enviromental Sciencies.	Sardine		Daily age reading criteria	
Campana SE, Neilson JD (1985) Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci., 42: 1014.	General			Otolith microstucture
Campana SE (2001) Accuracy, precision and quality control age determination, including a review of the use and abuse of age validation methods. J. Fish Biol., 59: 197-242	General		Methods to estimate age with its precision and accuracy	Compilation of values of CV and APE for using in age estimations by otoliths
Campana SE, Moksness E (1991) Accuracy and precision of age date estimates from otolith microstructure examination. ICES J. Mar. Sci. ,48:303-316			Determinate the accuracy of estimated ages by otoliths	General recommendations for otolith microstructure studies
Campana SE, Jones CM (1992) Analysis of otolith microstructure data. In: Otolith Microstructure Examination and Analysis (Stevenson DK and Campana S E, eds). Canadian Special Publications in Fisheries and Aquatic Sciences, 117: 73–100.	General			
Cermeño P *et al*. (2003) Validation of daily increment formation in otoliths of juvenile and adult European anchovy. J. Fish. Biol.,62: 679-691	Anchovy	Bay of Biscay	Validation	Daily growth increment validation in the otoliths of anchovy juvenile and adult
Cermeño P *et al*., (2006) Juvenile European anchovy otolith microstructure Sci. Mar., 70(3): 553-557	Anchovy	Bay of Biscay	Growth pattern	Anchovy Otolith microstructure

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
Cermeño P (2007) Analysis of the microstructure of the otoliths for growing and recruitment. Application to the European anchovy (*Engraulis encrasicolus*) in the Bay of Biscay. PhD thesis, University of the Basque Country, 139 pp.	Anchovy	Bay of Biscay	Growth pattern and validation	Anchovy otolith microstructure and validation of anchovy juvenile
Cermeño P *et al*. (2008) Setting up interpretation criteria for ageing juvenile European anchovy otoliths. Sci. Mar., 72: 733-742	Anchovy	Bay of Biscay	Reading criteria. Interpreta tion of growth pattern.	Daily growth increment interpretation criteria. GRB criteria is applied
Cotano U *et al*. (2008) Distribution, growth and survival of anchovy larvae (*Engraulis encrasicolus* L.) in relation to hydrodynamic and trophic environment in the Bay of Biscay. J. Plankton Res., 30: 467-481.	Anchovy	Bay of Biscay	Abiotic and biotic factors and Growth	
Dulèiæ J (1995) Estimation of age and growth of sardine, *Sardina pilchardus* (Walbaum, 1792), larvae by reading daily otolith increments. Fish. Res., 22: 265–277	Sardine	Adriatic Sea		
Dulèiæ J (1997) Growth of anchovy, *Engraulis encrasicolus* (L.), larvae in the Northern Adriatic Sea. Fish. Res., 31: 189–195.	Anchovy	Adriatic Sea		
Dulèiæ J, Kraljevic M (1996) Larval growth of anchovy, *Engraulis encrasicolus* (Fish) in the eastern Adriatic Sea. Vie Milieu, 46: 73–78.	Anchovy	Adriatic Sea		
García el al. (1998) Daily larval growth and RNA and DNA content of the NW Mediterranean Anchovy *Engraulis encrasicolus* and their relations to the environment. Mar. Biol., 166: 237-245.	Anchovy	NW Mediterra nean	Relation of larval growth and condition with environm ental variables	Methodology of extracting and mounting the otoliths Example of growth pattern in anchovy
García *et al.* (2003) Contribution of larval growth-rate variability to the recruitment of the Bay of Malaga anchovy (SW Mediterranean) during the 2000-2001 spawning season. Sci. Mar., 67 (4): 477-490	Anchovy	SW Mediterra nean	Relation of growth rates and recruitme nt	Methodology of extracting and mounting the otoliths Examples of growth pattern in anchovy

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
García *et al*., (2006) Field comparison of sardine post-flexion larval growth and biochemical composition from three sites in the W Mediterranean (Ebro river coast, bays of Almería and Málaga). Sci. Mar., 70(2): 79-91	Sardine	W Mediterranean	Relation of larval growth and condition with environmental variables	Methodology of extracting and mounting the otoliths Examples of growth pattern in sardine
Hernández C *et al*. (2009) Preliminary results on first check validation in European anchovy (*Engraulis encrasicolus*) otoliths. Working Document to ICES Workshop on Age reading of European anchovy (WKARA). Mazara del Vallo, Italy, 9–13 November 2009	Anchovy	Bay of Biscay	Validation	Check validation
Hernández *et al*. (2010) Impact of environmental factors on survival of juvenile European anchovy (*Engraulis encrasicolus*) in the Bay of Biscay: implications for recruitment. ISOBAY-XII International Symposium on Oceanography of the Bay of Biscay. 3-6 May 2010, IUEM Brest, France.	Anchovy	Bay of Biscay	Environmental factors	Effects of environmental factors on survival
Hernández C *et al*. (2012) Growth of European anchovy (*Engraulis encrasicolus*) in the Bay of Biscay (NE Atlantic) under different environmental factors: implications for recruitment. ISOBAY-XIII International Symposium on Oceanography of the Bay of Biscay. 11-13 April 2012, Santander, Spain.	Anchovy	Bay of Biscay	Environmental factors	Effects of environmental factors of growth
ICES (2010) Report of the Workshop on Age reading of European anchovy (WKARA), 9-13 November 2009, Sicily, Italy. ICES CM 2009/ACOM:43. 122 pp.	Anchovy		Age determination	Recommendations to perform studies on micro-increment daily growth as a validation tool for the first annual ring.
Jones CM (1992) Development and application of the otolith increment technique. In D.K. Stevenson and S.E. Campana (eds.) Can. Spec. Publ. Fish. Aquat. Sci. 117.	General		Sample preparation	
La Mesa *et al*. (2009) Growth and mortality rates of European anchovy (*Engraulis encrasicolus*) in the Adriatic Sea during the transition from larval to juvenile stages. Fish. Res., 96: 275-290.	Anchovy	Adriatic Sea		

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
May AW (1965) The validity of otolith ages of southern Grand Bank cod. ICNAF Research Bulletin, 2: 19-24.	General		Verification	Methods of age determination
Meneses I (2003) Estimação de factores que Condicionam a Variabilidade do Recrutamento de Peixes na Costa Atlântica da Península Ibérica. PhD Thesis, Portuguese Institute for Fisheries and Sea Research, Lisbon, Portugal. Available at: http://www.inrb.pt/fotos/editor2/Tese_IsabelMeneses.pdf	Sardine	Portuguese Coast	Sample preparation and Growth pattern	Otolith removal and microstructure interpretation
Morales Nin B (1988) Age determination in a tropical fish, Lethrinus nebulosus (Foskal, 1775) (Teleostei: Lethrinidae) by means of otolith interpretation. Inv. Pesq. , 52(2): 237	General		Age determination	Age determination by measuring growth rings formed on sagitta otoliths
Morales-Nin B *et al*. (2010) Age determination of larval and juvenile small pelagics: the importance of a common protocol. Rapp. Comm. int. Mer Médit., 39.	Anchovy and sardine		Daily Age reading criteria	Common Protocolol
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.	Anchovy and sardine	Adriatic Sea		
Panfili M *et al*. (2010) Growth rates of early life stages of *Engraulis encrasicolus* and *Sardina pilchardus* in the Adriatic Sea (Italy). Rapp. Comm. int. Mer Médit., 39, 2010	Anchovy and sardine	Adriatic Sea		
Panfili J, Morales-Nin B (2002) Manual of Fish Schlerochronology (Panfili J, Troadec H, De Pontual H and Wright PJ, ed.). Brest, France: Ifremer-IRD.	General			Otolith examination and analysis
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	Sardine	Portuguese Coast	Period of first-feeding	Age = number of increments + 4 days(first-feeding period)
Regner S, Dulèiæ J (1990) Growth parameters of anchovy post-larvae in the Adriatic estimated from otolith growth rings. Biljeske–Notes Institut za Oceanografiju Ribarstvo Split, 76: 1–7.	Anchovy	Adriatic Sea		
Romanelli M *et al.* (2002) Growth and mortality of exploited *Sardina pilchardus* (Walbaum) larvae along the western coast of Italy. Fish. Res., 55: 205–218.	Sardine	Adriatic Sea		

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
SARDONE Protocol (for otolith interpretation). In WP 3 Final report of SARDONE: Improving assessment and management of small pelagic species in the Mediterranean., February 2010	Anchovy / Sardine	North Aegean Sea Adriatic Sea Gulf of Lions	Homogenizing methodologies for preparation and interpretation of otoliths among laboratories	Comparing growth of larvae and juveniles among seasons and areas
Schismenou E *et al*. (2013) Seasonal changes in otolith microstructure, growth and condition of anchovy late larvae explained with a hydrodynamic-biogeochemical model simulation. Mar. Ecol. Prog. Ser., 478: 197–209	Anchovy	North Aegean Sea		Seasonal growth of anchovy late larvae and relation to environment
Schismenou E (2012) Modern approaches in biology and ecology of reproduction and growth of anchovy (*Engraulis encrasicolus*) in the North Aegean Sea (PhD Thesis). In Greek with English abstract	Anchovy	North Aegean Sea		Seasonal growth of anchovy late larvae and juveniles-relation to environment
Secor DH *et al* (1992) Otolith removal and preparation for microstructural examination. In: Stevenson DK, Campana SE (eds) Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117:19–57.	General		Sample preparation	
Silva AV *et al*.(2012) Determinação da idade de juvenis de sardinha (Sardina pilchardus, Walbaum, 1792) a partir de características morfométricas dos indivíduos e dos otólitos Relat. > Cient. Téc. Inst. Invest. Pescas Mar, Sárie Digital, nº 53. 24p.	Sardine	Portugues e coast	Ageing methods	Relationship between age and otolith weight
Somarakis S, Nikolioudakis N (2007) Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolous*) in the northern Aegean Sea (eastern Mediterranean). Mar. Biol., 152: 1143-1158	Anchovy	North Aegean Sea	Growth	Growth of early larvae – relation to environment
Somarakis S *et al*. (1997) Fluctuating asymmetry in the otoliths of larval anchovy *Engraulis encrasicolus* and the use of developmental instability as an indicator of condition in larval fish. Mar. Ecol. Prog. Ser., 151: 191–203	Anchovy	North Aegean Sea		Recent otolith growth of anchovy early larvae – relation to environment
Stevenson DK, Campana SE (eds) Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117	General			Otolith microstructure examination and analysis

REFERENCE	SPECIES	STUDY AREA	ISSUE	RELEVANT INFORMATION
Uriarte A *et al*. (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.	Anchovy		Age determin ation	Protocol for Anchovy annual Ageing determination criteria
Waldron ME (1994) Validation of annuli of the South African anchovy, *Engraulis capensis*, using daily growth increments. ICES J. Mar. Sci., 51: 233–234.	Anchovy		Age validation	Methods for age corroboration. Otolith rejection procedures.
Waldron ME, Kerstan M (2001) Age validation in horse mackerel (*Trachurus trachurus*) otoliths. – ICES J. Mar Sci., 58: 806–813.	General		Age validation	Methods for age corroboration. Otolith rejection procedures.

4 Standardization of Material and methods for preparation of otolith thin sections in anchovy and sardine

Before the foreseen workshop on daily age reading, it has been considered useful to send a questionnaire (see Section 4.2) in which each laboratory should provide information on their method of preparation and interpretation of the anchovy and sardine otolith microstructure in order to standardize a protocol of material and methods for studies of daily growth of these species, which took place during the workshop.

4.1 Species and areas

The species, life stages and areas are summarized in Table 4.1 by country and laboratories, where studies of anchovy and sardine daily growth are performed, as well as the use of otolith microstructure data and experience in other species are also detailed.

Daily growth studies of anchovy and sardine are currently carried out in different European laboratories from South European Atlantic and Mediterranean Sea, principally to analyse the effects of environmental parameters on growth and survival, and thus to understand the factors affecting recruitment processes of these species. Some laboratories also perform validation studies of these species.

4.2 Review Material and techniques

Based on the questionnaire delivered to WKMIAS from institutes prior to the workshop, the group reviewed the results and compiled the information about the material and techniques the different countries/laboratories use for storage before and after preparation, and the types of resins and saws used. Table 4.2 condensing the preparation method used by species and area from all countries/laboratories.

The main result is that the two species are treated using the same procedures by laboratories within the same area and among areas. However, a range of different materials for storage before treatment and different ways of making the thin section were applied. These differences does not necessarily imply unwanted bias regarding the precision of the age reading, however special attention should be with the otolith polishing, as there may be problems of over-under polishing, and this could influence the age readings estimation and introduce bias based on the method of preparation.

The table produced during the WKMIAS is an essential tool for standardization of the procedures applied for each species.

4.3 Review Method image process

The ability to readily acquire images of otolith micro structure and calibration of all acquired images with a scale bar is essential to ensure that images of good quality are available and thus avoid bias in estimates of the daily age readings.

Table 4.3 shows the image processing software, type of cameras and calibration images used by species and area from all countries/institute. This is a summary of the questionnaire delivered to WKMIAS from Institutes prior to the workshop. Several software to acquire and process the images and also to interpret and measure of micro increments are used. In some Institutes, anchovy and sardine image otoliths are read first in vivo.

Table 4.1 Species, stage of life and area by country and institute/laboratory, where daily growth studies of anchovy and sardine are made.

							Use of Data			
Country	Institute	Laboratory	Species	Stage of life	Area	FAO/ICES Division	Validation	Ecological & Environmental studies	Others	Experience in other species
France	IFREMER	Nantes	Anchovy	Larvae &juveniles	Bay of Biscay	27/ICES VIIIab		X		
			Sardine	Larvae	Bay of Biscay	27/ICES VIIIab		X		
Greece	HCMR	Crete	Anchovy	Larvae &juveniles	North Aegean Sea	GSA 22		X		
			Sardine	Larvae &juveniles	North Aegean Sea	GSA 22		X		
Italy	CNR-IAMC	Sicily	Anchovy	Juveniles	Strait of Sicily	Strait of Sicily		X		sardine juveniles/ Coryphaena hippurus juveniles/Merluccius merluccius juveniles
	CNR-ISMAR	Ancona	Anchovy	Larvae &juveniles	Adriatic Sea	GSA 17&18/		X		
			Sardine	Larvae &juveniles	Adriatic Sea	GSA 17&18/	X	X		
Portugal	IPMA	Lisboa	Sardine	Juveniles	North western Iberian Peninsula	27/ICES IXa	X	X		
Spain	IEO	Santander	Anchovy	Juveniles	Bay of Biscay	27/ICES VIIIabc	X	X		Mackerel larvae & juveniles/ Monkfish juveniles
		Vigo	Sardine	Juveniles	North western Iberian Peninsula	27/ICES IXa and Aquaculture	X	X		
		Malaga	Anchovy	Larvae	Western Mediterranean Sea	37.1.1,1.2,1.3		X		Tuna larvae & postlarvae/ Hake larvae & postlarvae
			Sardine	Larvae	Western Mediterranean Sea	37.1.1,1.2,1.3	X	X		
		Baleares	Sardine	Larvae &juveniles	Western Mediterranean Sea	37.1.1,1.2,1.3	For larvae	X		Tuna larvae & postlarvae
	AZTI	Pasaia	Anchovy	Larvae &juveniles	Bay of Biscay	27/ICES VIIIabc	X	X		Mackerel larvae & juveniles / Mesopelagic fish juvenile,

Table 4.2 Review material, techniques and preparation methods by species and areas (laboratory) to anchovy and sardine daily ageing.

					Storage before treatment					Preparation						
Country	Institute	Laboratory	Species	Calcified pieces	Dry/plastic moulds o vials	Borax-buffered formalin	Alcohol	Freezer	liquid nitrogen	Larvae	Juveniles	Cutting Plane respect to the fish	Resins	Saws /polisher machine	Storage after treatment	Document presenting the summary of the techniques/Referenc
France	IFREMER	Nantes	Anchovy Sardine	Otolith/sagittae	-	-	Larvae & juveniles	-	-	Direct reading	sanded and polished section	saggital	Thermic Joint: Crystalbond	manual	on glass slides	Allain et al., 2003
Greece	HCMR	Crete	Anchovy Sardine	Otolith/sagittae	-	Larvae	-	Juveniles	-	Whole mounted on glass slides	sanded/polished section	sagittal plane section	Thermoplastic resin: Crystalbond 509	Manual (grinding papers)	The glass slides with the otoliths are kept in boxes.	Schismenou et al., 2013
Italy	CNR-IAMC	Sicily	Anchovy	Otolith/sagittae	Juveniles	-	-	-	-	-	polished section	sagittal plane section	Entellan, Canada Balsam	Isomet and manual polishing	on glass slides	Arkhipkin, 1991
Italy	CNR-ISMAR	Ancona	Anchovy Sardine	Otolith/sagittae	Larvae & Juveniles	-	-	Larvae & Juveniles	-	Whole otolith mounted on glass slides	polished section	sagittal	Petropoxy 154 & Crystalbond 509	Manual polising metallographic (remet, Italy)	The glass slides with the otoliths are kept in boxes.	La Mesa et al., 2009
Portugal	IPMA	Lisboa	Sardine	Otolith/sagittae	Juveniles	-	-	-	-	-	polished section	sagittal plane section	Entellan®, Merck (ref: HX807787)	Imperial Lapping film, 3M ™ Electronics Markets Materials, Grade 0.3, 9 & 30 µm	microscope slides and stored in plastic boxes	Meneses, 2003
Spain	IEO	Santander	Anchovy	Otolith/sagittae	Juveniles	-	-	-	-	-	sanded section	sagittal plane section	Thermoplastic glue: Crystal Bond (mounting wax 40-8150)	Grinder-Polisher Buehler, model MetaServ2000.covered with a grinding paper (grit 3/5 µm) at 200-500 rpm	microscope slides and stored in specific cases.	Hernandez et al., 2009
Spain	IEO	Vigo	Sardine		Juveniles	-	-	-	-	-	polished section			Polishing machine Buehler, model Phoenix Beta, two smoothing-polish stations. Variable speed 30-600 rpm		
Spain	IEO	Malaga	Anchovy Sardine	Otolith/sagittae	-	-	-	Larvae	Larvae	Whole mounted on microscope slides with nail laquer	-	-	Nail laquer	-	microscope slides and stored in slides boxes	ICES, 2004
Spain	IEO	Baleares	Sardine	Otolith/sagittae	-	-	-	larvae & juveniles	larvae & juveniles	Whole otoliths mounted on microscope slides with Eukitt or thermoplastic cement	polished section	-	Eukitt or thermoplastic cement	manually with lapping film of different grains	Mounted on microscope slides with Eukitt or thermoplastic cement, organized and stored in order in slide boxes	ICES, 2004
Spain	AZTI	Pasaia	Anchovy	Otolith/sagittae	-	-	larvae	Juveniles	-	Whole mounted on glass slides with mail varnish	polished section	sagittal plane section	Crystalbond thermoplastic glue	Buehler Grider Polisher, 368 w	on slides in small plastic boxes	Aldanondo et al., 2008, 2010, 2011; Cotano et al., 2008; Cermeño et al., 2003.

Table 4.3 Review methods in images processing by species and areas (Laboratory) to anchovy and sardine daily ageing.

Country	Institute	Laboratory	Species	image capture/analysis sofware	Type of camera	Calibrated images	Calibration	Format
France	IFREMER	Nantes	Anchovy Sardine	TNPC 5.4	Digital Camera ; Réf : Sony XC 999 P	yes	x312; x500; x1250	im5, im6
Greece	HCMR	Crete	Anchovy Sardine	Image-Pro Plus 3.0, Media Cybernetics Inc., Silver Spring, MD, USA	Analogic: CCTV Panasonic camera (mod. Vw-cd20); digital: Q Imaging MicroPubliser 5.0 RTV	yes	x200, x1000 (nucleus)	Tiff
Italy	CNR-IAMC	Sicily	Anchovy	Leica IM50/Image pro Plus vers. 4.5.0.19	Leica DC200 digital color	yes	x100; x200; x400; x1000	Jpeg; Tiff
	CNR-ISMAR	Ancona	Anchovy Sardine	Leica LAS	Digital camera Leica IC80 HD	yes	x100; x250; x400; x630, X1000	jpeg; Tiff
Portugal	IPMA	Lisboa	Sardine	VISILOG 6.3/ TNPC 4	Sony digital camera DFY-SX910	yes	x1000 (nucleus) and x200	Tiff
Spain	IEO	Santander	Anchovy	Image mosaic (Visilog MACROS/VISILOG 6.4/TNPC 4.2	Video Camera. Sony Digital Interface-DFW-SX910	yes	x1000 (nucleus) and x100	im5, im6, tiff, jpeg, jp2
		Vigo	Sardine	TNPC 4, Image mosaic with Image J/ TNPC 4	Color Digital Camera: NIKON DS-2Mv & Sony DFW-SX910	yes	x1000 (nucleus) and x200	
		Malaga	Anchovy Sardine	*Live image* with OTO program in the past/ Actually *Live image* with Nikon ACT-2U and images capture with Image J program	Nikon Digital Sight DS-U1	yes	x1000	Tiff
		Baleares	Sardine	In the past, Live image with OTO program/ Actually images capture with Image J program	Digital cameras: MicroPublisher 3.3 RTV and a Leica DFC 495	yes	x1000 larvae/juveniles '	jpg, tiff, png, bmp, jpeg
	AZTI	Pasaia	Anchovy	Visilog, TNPC Software, v.3.2	Analogical black and white video camera (Sony, SPT-M328CE)	yes	x1000 (nucleus) and x100	im5

4.4 Standardization of protocols

Based on the review of the procedures and techniques used in each institute, a common protocol of material and methods for larvae and juvenile anchovy and sardine otolith microstructure was elaborated at the workshop as follows below:

Calcified pieces

- Sagitta otoliths have to be employed.
- Indistinctly right or left otoliths can be used.

Otolith storage before treatment

- Dry, alcohol buffered or freezed (liquid nitrogen or freezer).
- Larval samples could be stored in buffered formalin (pH>8.5) or with alcohol buffered with tris (pH has to be checked every 3 hours for 24h).

Preparation of sample

1.1) Larvae

- Whole otolith
- Embedding: nails lacquer or petropoxy or thermoplastic glue.

1.2) Juveniles: Preparation of thin section

- Cutting plane: sagittal plane
- Position of the sulcus : not relevant
- Embedding: resin or thermoplastic glue
- Grinding: Manually or semi-automatic grinding: Decreasing grain size paper (2000-4000 grid or in microns:30 to 0.3)
- Polishing: Allumina paste(1-0.3 micrometre), diamond suspension (1 micrometre) and Canadian Balsam

Image processing

- Oil immersion in every magnification to smooth out the images reducing imperfections.
- Calibration: Its mandatory enclosed the calibration what corresponding to the image. The different magnifications have to be calibrated

4.5 Alternative techniques for standardize the age estimation.

Age estimation by otoliths is paramount to fisheries science. Ages of around a million fish are estimated yearly by the interpretation of otoliths (Campana and Thorrold, 2001). This implies a meticulous process of extraction and mounting of otoliths. The complexity of the process consumes time and the interpretation of daily increments requires expert qualifications to obtain reliable results (Megalofonou, 2006). As a result, age determination in larval fish implies a high cost/benefit analysis per otolith (Bedford, 1983; Cardinale and Arrhenius, 2004; Francis and Campana, 2004). Moreover, one of the most common sources of error relies on the subjective criteria that an age reader may have. Subjectivity, together with the differences in preparation process of otoliths and the variability of the interpretation of the periodic changes shown in calcified structures are among the major sources of between different age reader´s age estimates (Boehlert, 1985; Campana and Moksness, 1991).

Application of alternative techniques in order to reduce the influence of these sources of error in age estimation process should be considered. One of these alternative techniques is the use of discriminant analysis for estimating age.

Discriminant function analysis allows classifying individuals of unknown origin into groups by using discriminant functions generated from a database of information of individuals of known origin (McGarigal *et al*., 2000). It was first used by Fletcher and Blight (1996) to determine age on the basis of somatic and otolith biometric measurements.

The objective of these techniques is construct a predictive model that allows to estimate age with a high percentage of correct assignments and low values for the precision estimators between direct and estimated ages (APE and CV) based on somatic and otolith biometry of larvae through an important reduction of subjectivity, increasing the repeatability of age readings and reducing cost/benefit ratio per otolith.

4.5.1 Age estimation by discriminant analysis in ANCHOVY larvae

During the workshop on Micro increment daily growth in European Anchovy and Sardine (WKMIAS 2013) results from "Estimating Daily Age of anchovy larvae using discriminant function analysis" were presented and included in the final report (J.M. Quintanilla and A. García WD 06, Section 10).

Anchovy larvae used for this study were collected in 2009 during the yearly MEDIAS (Mediterranean Acoustic Survey). Oblique plankton tows were undertaken with Bongo 60 and Bongo 90 ichthyoplankton sampling frames. The area covered by the survey encompassed the whole NW Spanish Mediterranean and part of SW Mediterranean (Alborán Sea).

Besides the age by direct readings, somatic (Standard length and Dry weight) and otolith biometric (Area, Perimeter, Radius, Inc-Med, Size Width and Size Length) variables were obtained.

Larvae from NW Mediterranean (NWM) were then divided into two groups with the sole premise in the selection process of maintaining in both groups the size distribution of the original population.

NWMTR (n LARVAE = 50; n OTOLITHS = 75)

NWMTS (n LARVAE = 41; n OTOLITHS = 71)

The group NWMTR (training sample) is used to calculate the discriminant functions for the prediction of otolith increment counts. On the other hand, NWMTS (test sample) was used to test the differences between the predicted increment counts of the model with the increment counts.

Prior to calculating the discriminant functions, the somatic and biometric variables of the otoliths were analysed to find which variables correlate best with the larval increment counts to include in discriminant analysis. To this purpose, a series of stepwise multiple linear regression (SMLR´s) was applied after colinearity of the variables included were tested (Table 4.5).

Afterwards, discriminant functions were applied for estimating increment counts and to determine the model's precision the proportion of correct increment counts assignments, the average percentage error (APE), the coefficients of variation means (CV) were calculated (Table 4.4)

Table 4.4 Results from SMLRs analysis for the full and filtered model with explained variance (R^2), significance (indicates p<0.01) and the variables selected to be included in DDFF analysis.**

VARIABLES INCLUDED IN SMLRs	MLR	R2	p	VARIABLES SELECTED BY SMLRs
Length, Weight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL	Forward	97%	**	Mean Inc-W, Radius and Area
	Backward	96%	**	Mean Inc-W, Radius, Area and Size W
Length, LogWeight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL	Forward	97%	**	Mean Inc-W, Radius, Perimeter and Area
	Backward	96%	**	Mean Inc-W, Radius, Area and Size W
Log(Length,Weight, Mean Inc-W, Radius, Area, Perimeter, SizeW and SizeL)	Forward	97%	**	LogInc-Med and LogRadius
	Backward	97%	**	LogInc-Med and LogRadius
Length, Weight, Area, Perimeter, Size W and Size L	Forward	81%	**	Perimeter and Area
	Backward	80%	**	Perimeter
Length, LogWeight, Area, Perimeter, Size W and Size L	Forward	81%	**	Perímeter and Area
	Backward	80%	**	Perimeter
Log (Length,Weight, Area, Perimeter, Size W and Size L)	Forward	81%	**	LogPerimeter
	Backward	81%	**	LogPerimeter

Table 4.5 Results from DDFF analysis for the full and filtered model with % Assignment for each error range; Average percent error (%) and Coefficient of variation (%).

VARIABLES INCLUDED IN DDFF	% ASSIGNMENT ± 0	± 1	± 2	± 3	> ± 3	APE MEAN	% CV MEAN
Mean Inc-W+Radius+Area	48	96	100	100	100	1.68	2.38
Mean Inc-W+Radius+Area+SW	54	97	100	100	100	1.46	2.07
Mean Inc-W+Radius+Perimeter+Area	51	99	100	100	100	1.52	2.14
LogRadius+LogInc-Med	38	94	99	99	100	2.03	2.88
Perimeter+Area	23	63	83	96	100	3.92	5.54
Perimeter	23	66	83	99	100	3.66	5.18
LogPerimeter	25	65	87	96	100	3.65	5.16
Perimeter2+Area2	21	75	90	99	100	3.33	4.71
Perimeter2	24	62	79	94	100	4.10	5.79
LogPerimeter2	25	63	87	96	100	3.70	5.24

Taking into account which larvae are included for calculating the discriminant functions (training sample) and those in which are applied (test sample), three scopes of applicability were considered: northwestern Mediterranean Training Sample group (NWMTR) to estimate the age of northwestern Mediterranean Test Sample larvae (NWMTS), northwestern Mediterranean Training Sample group (NWMTR) to estimate the increment counts of southwestern Mediterranean population (SWM) and Total northwestern Mediterranean (NWM) to estimate the age of southwestern Mediterranean population (SWM) (Figure 1).

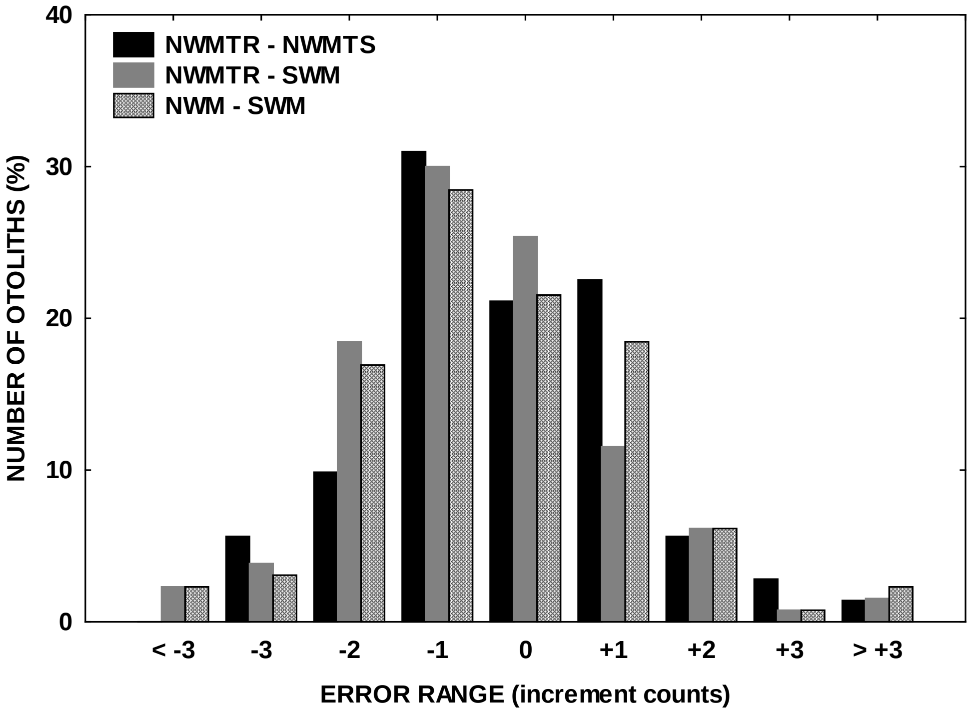


Figure 1 Differences between increment counts obtained by direct readings and estimated by the model Perimeter²+Area² for each otolith in the three scopes of applicability considered (NWMTR in NMWTS, NWMTR in SWO and NWM in SWO).

As summary, the main results of this study were:

- The predictive model (Perimeter2+Area2) estimates correctly the age of the otoliths in 75% of the cases assuming ± 1 day of error increasing to 90% assuming ± 2 days of error.
- APE and CV are systematically low indicating the precision of the count estimates
- Only these otolith variables were selected by MLR analysis because otolith measurements showed much better correlations with age than somatic variables.
- The discriminant functions obtained from one population can be applied to estimate the age of larvae from another population of the same species providing we have the same length distribution (even if population growth differences are observed between them).
- The results (% assignments, APE and CV) remain the same even if we duplicate the number of otoliths in the training sample to calculate the discriminant functions.
- No significant differences were observed between the estimated ages from direct readings and those estimated by the model.

In conclusion, the proposed method:

- Reduces the subjectivity of readers compared with direct reading methods.

- Is time saving as it does not require as much increment readings.
- Reduces the cost/benefit ratio for ageing studies in fish larvae.

4.5.2 Age estimation by discriminant analysis in SARDINE Juveniles

Data on the age, growth and birthdates of juvenile fish are important to investigate periods when environmental conditions are most favourable to recruits survival (Baumman, 2008). Otolith and fish morphometric characteristics were shown to be good predictors of age in adult fish of several species (Pawson, 1990; Flecher, 1991, 1995; Fossen, *et al*., 2003; Lou *et al*., 2007; see review by Francis and Campana, 2004). The utility of otolith and individual morphometric characteristics to predict the daily age in juvenile stage life has being poor investigated. Such knowledge is most relevant in the case of small pelagic fish, such as sardine, that show wide interannual fluctuations in recruitment (Stratoudakis *et al*., 2007; ICES, 2011).

During the workshop, a study on the determination of sardine age from the morphometric characteristics, carried out in the Portuguese coast, was presented (Silva *et al*., 2012). This study aims to identify a relationship to predict the age, in days, of sardine juveniles from otolith (diameter and weight) and individual (total length and weight) morphometric characteristics. Several possible relationships are evaluated, about their ability to predict the age distribution and the growth parameters derived from observed ages and the age distribution is then compared between the ages predicted by the model and ages read by increment counts.

Monthly or bimonthly samples of juvenile sardines (age class 0) were collected between May 2004 and January 2005 from the landing harbours off the northwest coast of Portugal (Table 4.6). Total length (TL, nearest 0.1 cm) and total weight (Wt, nearest 0.01g) were recorded, selected from the modal class and tails (5 each) of the length frequency distributions of each of the samples. Also Otolith diameter (OD), the distance between the furthest edges of anterior and posterior zones, passing through the nucleus (Figure 2), and Otolith weighed (OW, nearest 0.0001g) was measured.

Table 4.6 Monthly samples of sardine juveniles and number of analysed fish per each sample. MAT – Matosinhos; POV – Póvoa do Varzim

Date	Harbour	Fish length [range] (cm)	Training sample	Test Sample
20-05-2004	POV	[4.5-11.5]	12	
27-05-2004	POV	[8-10]	7	9
04-06-2004	POV	[8-19.5]		10
28-06-2004	POV	[9-12.5]	6	6
1-07-2004	MAT	[4.3-9.2]		
20-07-2004	POV	[8.5-13.5]	9	3
26-07-2004	MAT	[9.5-12.5]		
24-08-2004	MAT	[10-14.5]	4	8
06-09-2004	MAT	[11 – 14.4]		
23-09-2004	MAT	[11-14]	5	8
16-11-2004	POV	[11.5-14.5]	3	2
19-11-2004	MAT	[11-14.5]	4	10
10-01-2005	MAT	[9-13]	8	5
TOTAL			58	61

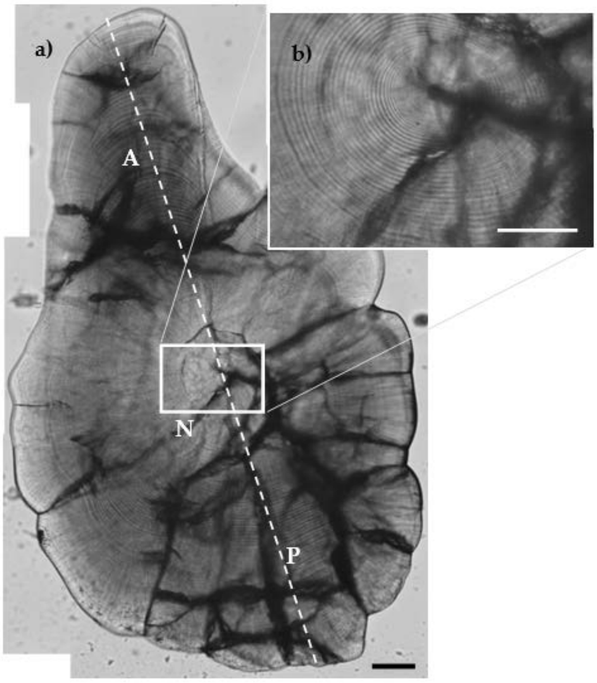


Figure 2 Composite micrograph of the whole otolith of a juvenile sardine otolith assembled from 26 micrographs of parts of the otolith (scale bar=100 μm) (a) and micrograph of the central area of the otolith. Amplification= 200x (scale bar=50 μm) (b). A – Anterior edge; P – Posterior edge; N - Nucleus. Dashed line indicates otolith diameter. Amplification= 400x.

Otolith daily growth rings were counted on a sample of 119 individuals (selected from the modal and extremes length classes) spanning the study period as evenly as possible, and divided in two samples; train sample (TrS; n= 58) and test sample (TeS, n=61). The TrS were used to investigate the relationships of age with the individual and morphometric characteristics and predict the best model. On the other hand, TeS was used to test the differences between the predicted increments counts with the increment counts.

Otolith characteristics correlate best with juvenile sardine ages (Table 4.7). A multiple linear regression model was fit to Age using the four morphometric characteristics as additive effects. To produce the final predictive equation, the multiple regression model was simplified by searching, in a stepwise procedure, for the combination of variables that minimize the Akaike Information Criterion (AIC). Only *O*d was selected. Age was ln transformed to comply with assumptions of normality and variance homogeneity.

Table 4.7 Pearson correlation coefficients and variation infraction factor (VIF) for age and fish, otoliths morphometrics

	*T*W	*O*W	*O*D	AGE	VIF
*T*L	0.96	0.92	0.95		27 101
*T*W		0.87	0.87	0.85	21 238
*O*w			0.97	0.90	9170
*O*d				0.91	4914

The morphometric variables were strongly collinear (Variance Inflation Factor (VIF)>5 in all pairwise combinations) and so a simple linear regressions models were fit to each combination of age - morphometric variable. In this case, the cubic root of *T*w and *O*w were used in the models to obtain linear relationships with ln(Age). According to AIC and the resulting model was then applied the TeS and model estimated ages generated by the ages read in increment daily counts and predicted by the model was not significantly different (Kolmogorov–Smirnov test, p= 0.9963, D=0.1667; Figure 4.3, Table 4.8).

Table 4.8 Parameters of single models of biometric measurements of fish and otoliths with age

Ln(Age) vs	a	b	R^2	p	AIC
*O*d	5.51e-4	4.17	0.86	<0.01	-104.8
⅓*O*w	1.72	3.84	0.85	<0.01	-102.8
*T*L	0.11	3.99	0.79	<0.01	-83.3
⅓ *T*W	0.55	4.03	0.78	<0.01	-98.9

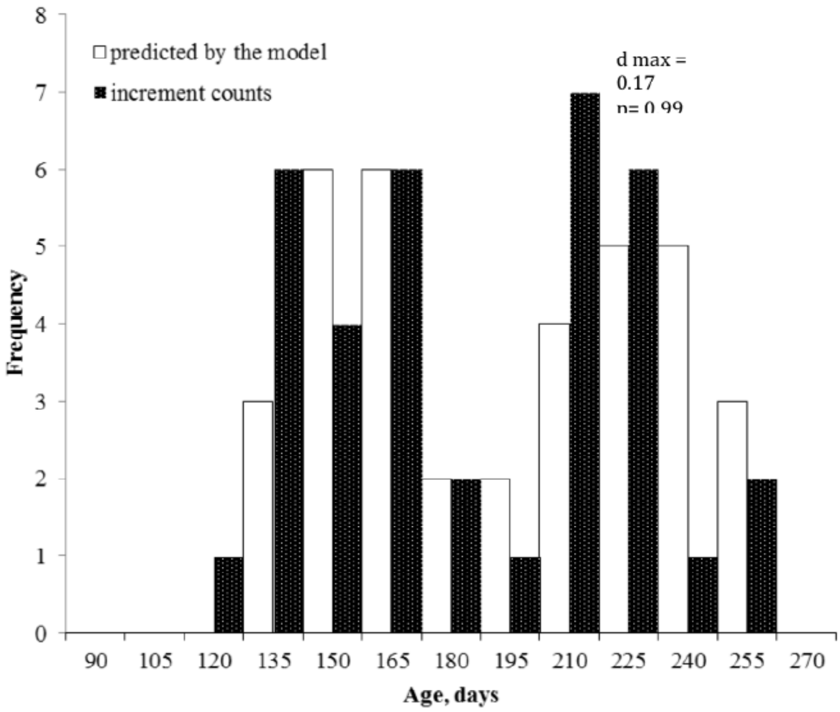


Figure 3 Comparison of population daily age structure of *Sardina pilchardus* produced from: (■) direct reading in otolith increment counts and (□) age derived from otolith diameter regression. Regression equation from table III (dmax and probability values from K-S test).

As summary, the main results of this study were:

- Otolith measurements showed better correlations with age than somatic variables;
- Only otolith diameter explains 86% of the variability of daily age juveniles;
- No significant differences were observed between the estimated ages from direct readings and those estimated by the model;

- The collection of otolith morphometry is comparatively simpler and faster to obtain and may complement traditional otolith reading to obtain a larger volume of data, significantly reducing costs and time for ageing and recruitment studies.

This work is still ongoing and therefore the results must be carefully considered.

5 Definition and standardization of reading criteria among areas

5.1 Review Reading criteria

For otolith daily increment interpretation two different criteria has been suggested: in the first one, known as group band reading (GBR), 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 one, 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. This criterion was further discussed in the framework of SARDONE project and its suitability for Mediterranean anchovy and sardine was tested. The final agreement was to apply this methodology (GBR) for all anchovy and sardine, irrespective of the season and geographical area (Morales-Nin *et al*., 2010; SARDONE protocol, 2010).

However, during this workshop some participants adopted the IMR criterion for anchovy juveniles in the Adriatic Sea and Strait of Sicily. This discrepancy was discussed again but it did not come to a final agreement. Therefore, it is strongly recommended to validate the IMR criterion in anchovy in these specific areas.

In addition, the microincrement counts are generally performed "in live", i.e. directly on slides by adjusting the focus along the counting path, when using IMR, and performed on fixed otolith images when using GBR (see the questionnaire).

5.2 Review Validation

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

5.3 Review Quality control

To test quality control of age estimates, most of laboratories from different countries developed internal (reading procedure) and external (planning otoliths exchanges and working groups) practices (see the questionnaire). The internal practice mainly concerned repeated readings made independently by either single or more readers, to check for precision in age estimates. Generally otoliths are discarded when error in reading precision is more than 5-10%. As far as external practices are concerned, laboratories from different countries (i.e. Spain, Greece and Italy) participated to the EU SARDONE project ("Improving assessment and management of small pelagic species in the Mediterranean") in 2007-2010, and planned the Workshop on Age reading of European anchovy (WKARA) in 2009 and the Workshop on Age reading of European Atlantic Sardine (WKARAS) in 2011.

5.4 Results from the otolith reading exercise in anchovy and sardine for the standardization of the adoption of ageing criteria

See the documents produced during the workshop.

5.5 Definition and standardization of age assignment protocol

Taking into account the results from the otolith reading exercises in anchovy and sardine carried out before and during the workshop, as well as the past experience acquired in laboratories from different countries, a common age assignment protocol has been defined as follows, separated for each species and early stage of development.

<u>Anchovy</u>

- Larvae
- The whole otolith should be read at 1000x magnification along the maximum growth axis.
- Juveniles
- Readings have to be done along the post-rostrum axis, which is generally the maximum growth axis of sagittal otoliths.
- The otolith radius has to be measured along the post-rostrum axis.
- The otolith nucleus should be read at x1000 magnification. For the rest of the otolith, it depends on the reading criteria: if the GBR method is applied, the otolith should be read at x 100 or -x200 magnification. If the IMR criterion is followed, x400 magnification should be used. Close to the otolith margin, at the beginning of the formation of the first translucent zone, the magnification should be increase at x630.
- There was not an agreement in reading interpretation criteria. For some readers (Italy), both age interpretation methods (GBR and IMR) are considered valid: it depends on the distribution area. For the rest of the participants (Spain, Portugal and Greece), the GBR criteria is the most reliable method for ageing anchovy. As it can be observed in Table 5.5.1 the agreement varies among areas and readers.
- When unreadable areas are spread over more than 10% or more of the reading path the otolith should be rejected.
- The otolith has to be read at least two times, possibly by different readers, from the primordial to the otolith margin and vice versa.
- When discrepancy among readings is more than 10%, a third reading should be carried out. If the discrepancy persists, the otolith should be discarded.
- Counting of otolith microincrements has to be started from the hatch check and 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).

Table 5.5.1:

LIVE STAGE	AREA	READING INTERPRETATION		MAGNIFICATION			
		GBR (%AG.)	IMR (% AG.)	200X (%)	400X (%)	630X (%)	1000X (%)
LARVAE	WESTERN MEDITERANEAN	100					100
JUVENILES	ADRIATIC SEA	77	23	77	23		
JUVENILES	BAY OF BISCAY	100		100			
JUVENILES	NORTH AEGEAN SEA	100		100			
JUVENILES	STRAIT OF SICILY	67	33	67	33		

<u>Sardine</u>

- Larvae
- The whole otolith should be read at 1000 magnification along the maximum growth axis.
- Juveniles
- Readings have to be done along the post-rostrum axis, which is generally the maximum growth axis of sagittal otoliths.
- The otolith radius has to be measured along the post-rostrum axis.
- The otolith nucleus should be read at 1000 magnification, while the rest of the otolith at x200 or x400. It depends on the reading interpretation criteria.
- The age interpretation method is the GBR in all areas investigated, except for the Adriatic Sea where IMR is adopted (Table 5.5.2).
- Counting of otolith microincrements has to be started from the hatch check, which is located at 5-7 µm of distance from the primordium (Alemany and Alvarez 1994).

Table 5.5.2:

LIVE STAGE	AREA	READING INTERPRETATION		MAGNIFICATION			
		GBR (%AG.)	IMR (%AG.)	200X (%)	400X (%)	630X (%)	1000X (%)
LARVAE	WESTERN MEDITERANEAN	100					100
JUVENILES	ADRIATIC SEA		100	60	40		
JUVENILES	ATLANTIC (AQUACULTURE)	100		100			
JUVENILES	NORTH AEGEAN SEA	100		100			
JUVENILES	NORTH PORTUGAL COAST	100		100			
JUVENILES	STRAIT OF SICILY	100		90	10		

6 Validation of the first annulus in young of the year anchovy and sardine

6.1 Anchovy Corroboration

IEO scientists presented a paper which corroborates the position of the first false ring (check) for anchovy in the Bay of Biscay (Hernández, C. *et al*.; 2013). This study arises from the two recommendations given in the Workshop on Age reading of European anchovy, WKARA 2009, due to the difficulties encountered by various experts readers in annual age to determinate between first annual ring and the check formed before its first winter ring.

Two methods were used, in the first, age was determined by identifying and measuring growth rings formed on sagitta otoliths (Morales-Nin, 1988). In order to support the identification of the first annual ring, the otolith radius of the first hyaline ring was measured and used as a gauge for exclude the first check in ageing older individuals.

In the second, a method for age corroboration was used by means of the otolith microstructure and fish ages were determined by daily increment counts (Campana *et al*., 1985; Campana, 2001). Total number of daily increments in otoliths was counted to test whether identified macroscopically hyaline area is, in fact, a check or the first annulus (Waldron *et al*., 1998, Waldron *et al*., 2001).

We compare the results of age determination using whole otoliths with those determined through counts of daily increments in an attempt to corroborate the above method.

6.1.1 Material and methods

6.1.1.1 Analysis of whole otoliths

A total of 358 otoliths were collected from *Engraulis encrasicolus*, in the total length (TL) range 11.7-20.5 cm. The fish were caught in the Bay of Biscay between 2010 and 2011. The radius (µm) was measured across the widest part of the otolith, along the same axis used to estimate the age of the fish, the presumed check and annual growth rings distances were measured between the core and the inner edge of hyaline rings (Figure 4), although this is usually measured between the outer edges, we considered more convenient to measure in this way, as in many of the specimens studied had not formed completely hyaline ring and because the inner edge of the hyaline ring is usually well marked in otoliths and can be measured accurately. Once all the increments (hyaline rings) on otoliths have been identified, an expert reader established the age of fish by counting the number of seasonal increments on an annual basis (Uriarte *et al*., 2002).

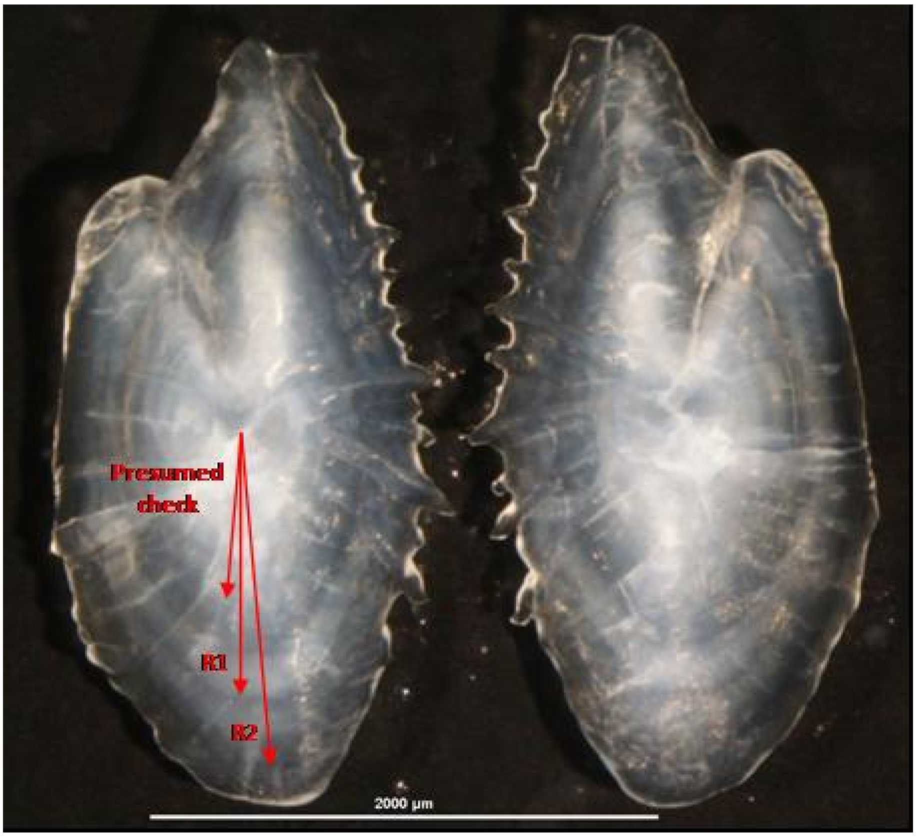


Figure 4. Measurements taken from anchovy otoliths. Presumed check= false ring, R1= 1st annual hyaline ring, R2= second annual hyaline ring.

6.1.1.2 Analysis of otolith sections

To identify if the formation of this first ring is a check or an annulus daily increment structure was examined in a total of 6 otoliths from fish (TL range 13.0 -14.5 cm) caught in March, April and June in which the expert age reader determined the presence of a presumed check . Daily growth increments were examined at 1000x magnification for the core area and the outer part of the otolith was analysed at x100 and x200 magnification. The criterion named Group Band Reading (GBR) was used for daily increment interpretation (Cermeño *et al.*, 2003, Cermeño *et al.*, 2008). Growth increments were counted from the core, beginning at hatch increment (Aldanondo *et al.*, 2008), to the outer edge of otolith. Otolith radius, presumed first check and 1st annual ring were measured along the same axis. Each otolith was read at least 2 or 3 times until a consistent increment count was obtained. When error in reading precision was more than 10%, a third reading was taken. If the discrepancy persisted, the otolith was discarded. Also an otolith was rejected if the primordium was not visible or if more than 10% of the increment count was only possible by interpolation (Waldron *et al.*, 1998, Waldron *et al.*, 2001).

6.1.2 Results

6.1.2.1 Analysis of whole otoliths

The results showed that increments widths have a normal distribution (Kolmogorov–Smirnov test, Presumed Check, R1, R2 and R3 values p > 0, 05) with a falling rate of otolith growth with age (Figure 5). This linearly decreasing interval between increments is a verification criterion that forms the basis of age estimation (May, 1965).

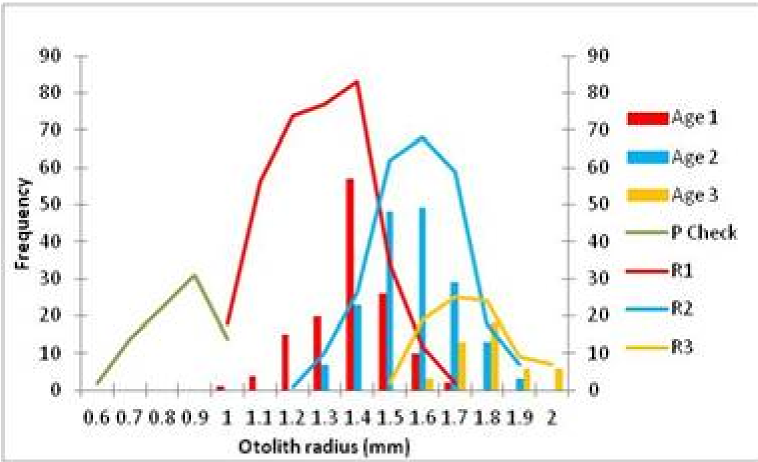


Figure 5. Frequency distribution of rings distances presumed check, R1, R2, R3 and tree annual age ring distances.

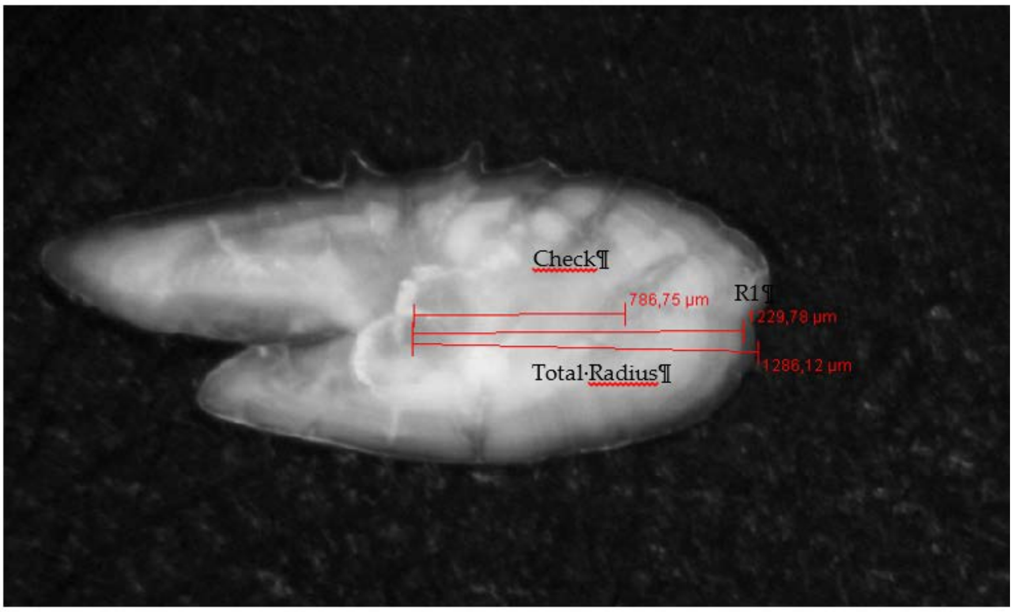
In cases where the distance from the core to the first visible ring was < 852± 100 µm, this ring was assigned by the reader as a presumed check. Based on the measurements made, a guideline table for age interpretation was established (Table 6.1).

Table 6.1 Guidelines for age estimation. The distance between the core to the beginning of the presumed check and winter rings (µm): average, standard deviation (s.d.), and minimum and maximum values observed.

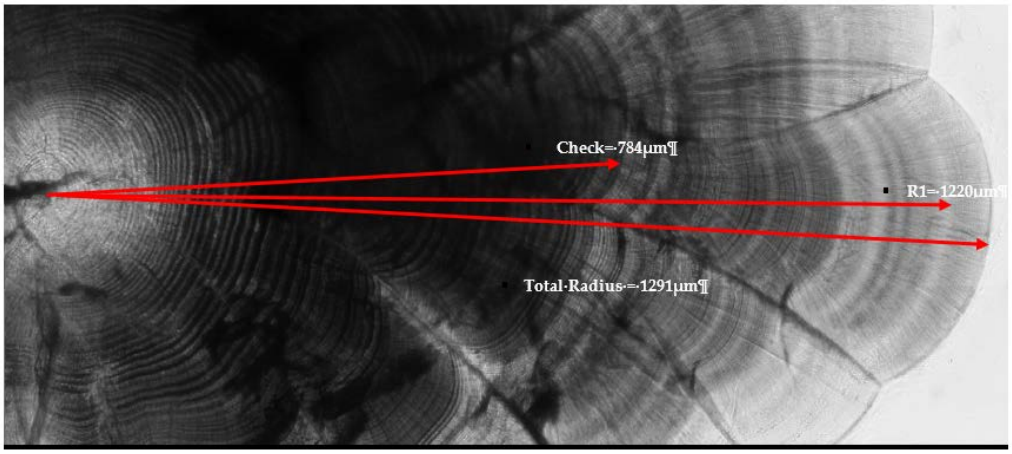
Rings	Average (µm)	s.d	Minimun (µm)	Maximun (µm)
Presumed Check	852	100	593	999
R1	1295	147	1000	1675
R2	1589	133	1202	1936
R3	1750	124	1523	2047

6.1.2.2 Analysis of otolith sections

The growth of the entire sequence of opaque and translucent zones was analysed for a subset of selected otoliths in which the daily increment structure to the check and to the 1st annual ring (R1) was clear (Figure 6 a, b). To ensure good-quality results, rigorous rejection procedures were applied to the otoliths, for this reason so far only six otoliths have been analysed and read with confidence (Waldron *et al.*, 1998, Waldron *et al.*, 2001).



(a)



(b)

Figure 6. Measurements taken from the same anchovy otolith, (a) whole otolith and (b) otolith section. Check= false ring, R1= 1st annual hyaline ring and Total Radius. (Daily increments to Check = 73 days, Daily increments to R1=185 days, Total Daily increments=211 days)

For otoliths analysed the average distance between the core to the beginning of the check was 800 ± 69 µm at a mean age of 94 ± 27 days (Table 6.2 and 6.3)

Table 6.2 Otolith radius to Check, R1 and Total, obtained by daily increment counts.

	1	2	3	4	5	6	Average (µm)	s.d.
Radius to Check (µm)	915	800	714	784	756	833	800	69
Radius to R1 (µm)	1396	1424	1200	1220	1400	1496	1356	119
Total Radius (µm)	1632	1774	1596	1291	1520	1637	1575	162

Table 6.3 Otolith Ages (days) to Check, R1 and Total, obtained by daily increment counts.

	1	2	3	4	5	6	Average (days)	s.d.
Increments to Check (days)	138	117	74	73	82	80	94	27
Increments to R1 (days)	276	279	124	185	282	284	238	68
Total Increments (days)	353	394	225	211	309	338	305	73

6.1.3 Conclusion and Discussion

The results obtained through the analysis of otolith microstructure indicated that the hyaline zone macroscopically identified as a check is not an annual growth area because they had less than 365 daily increments in otoliths before deposit of this ring.

If we analyse the otolith macroscopically we found that the first check was 852 ± 100 µm and if we look through the otolith microstructure it is at an average distance of 800 ± 69 µm, the observed difference in both cases, apart from the difference in the number of specimens analysed is due mainly to the difficulty in identifying macroscopically the exact position of the nucleus, it also would explain the high difference between maximum and minimum ring distances (Table 6.1.1.2.1). One recommendation to solve this problem would be to place one of the otoliths with the proximal face up (sulcus face) when the reader is measuring otolith rings distances, as the core is best located on this side. Thus we could obtain more accurate measures and average distance values would be more fitting.

This study has confirmed that the estimated ages read using whole otoliths in relation with those determined through counts of daily increments are generally correct up to the age of one year, supporting that the reader can distinguish correctly between rings and false rings and fish can be assign to the correct year class.

Based on the corroboration of age estimates presented here two recommendations to annual readers for ageing routines have been established (see Annex 3: Recommendations).

1-It is recommended to anchovy annual readers to measure the distances between the core and the inner edge of the hyaline rings, it is usually well marked in the otoliths and can be measured accurately.

2-From the validation of daily ring formation in anchovy larvae and juveniles (Cermeño *et al*., 2003; Aldanondo *et al*., 2008), and corroboration of the position of the first hyaline ring (check) formed before their first winter annual ring, through the counting of micro-increments, is suggested to measure 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.

6.1.4 Anchovy Validation

Otoliths contain information about the age and growth history in marine fish, both on daily and annual level. On a daily level, otolith microstructure analysis provides information about birth-date distributions and previous growth history of larvae and juveniles. This technique is based on two main assumptions that need to be validated: a daily periodicity of increment formation and a strong relationship between otolith and fish growth. For European anchovy, daily increment deposition has been previously validated for anchovy larvae (Aldanondo *et al.*, 2008) and juveniles (Cermeño *et al.*, 2003) and the relationship between otolith size and fish size has been studied (Aldanondo *et al.*, 2008, 2010).

On an annual level, otolith macrostructure analysis allows fish age determination, which is fundamental to an understanding of their population dynamics. It is assumed that the annual growth increment, known as annulus, is laid down at regular interval following a seasonal cycle. The annulus is composed of an opaque and a translucent band, which appear to differ optically under transmitted light. The opaque band formation is related to fast growth period, whereas the translucent band or "winter ring" is generally associated with a period of slow growth rate. However, in order to obtain reliable age estimates it is also necessary to validate annual increment deposition in otoliths. In the case of European anchovy, this validation study has not been previously done.

During the workshop, an annual increment validation study was presented (WD 01). In this study, the first annulus was validated using daily increments counts. According to that, in the case of European anchovy in the Bay of Biscay, the first opaque band is completed in October-November, whereas the translucent band is formed by March-April. (Figure 7).

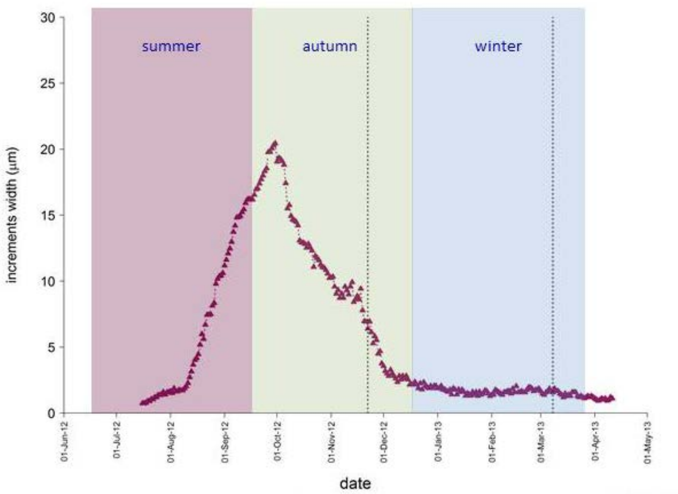


Figure 7 Daily increment width at age of anchovy juvenile sampled in April 2013. Vertical dashed lines represent the translucent band.

The main item in the discussion was the question whether the first annual increment validation was restricted to the anchovy population concentrates in the Bay of Biscay. In this sense, some participants suggested that it would be interesting to validate the annual increment deposition in other anchovy populations.

6.2 Sardine Corroboration

Corroboration of the identification of first *annulus* in young of the year sardine in Adriatic sea.

Aiming at corroborating the identification and position of the first annulus in young of the year sardine, daily Micro increment counts were carried out on 1 year old fish based on annulation pattern.

Preliminarily, four otoliths of sardine were examined. Individuals selected for the study ranged between 11.5 and 13 cm and were caught in October-December 2012 in Adriatic Sea.

The otolith microstructure was analysed through daily growth increments count using a light microscope at 400x magnification along the postrostrum axis.

The characteristics of otoliths analysed were summarized in the Table 6.4.

For the otoliths analysed the average distance between the primordium to the first annual ring (measured at the end of hyaline ring) was 885.7± 22.1 µm. Below this distance, all hyaline rings should be considered as false rings or checks (Figure 8).

This preliminary study shows that age of fish based on the annulation pattern (annuli) is corroborated by daily increments counts, providing indication on the minimum distance from the primordium to correctly distinguish false rings or checks. Additional samples of 1 year old fish will be analysed to better identify the timing and the position of the first ring (annulus), being one of the main sources of error affecting ageing precision.

Table 6.4 Otolith Sardine samples.

Sample	AGE	TL (cm)	Total daily increments (average)	s.d.	Total Radius (µm)	R1 1st annual ring (µm)
97038S	1	11.5	293	4	1023.605	869
96830S	1	13.0	308	5	1010.796	866
96866S	1	12.5	345	6	1069.208	912
97447S	1	12.5	382	3	1165.716	896

Figure 8 Measurements taken from sardine otoliths. C=check, R1=1st annual hyaline ring.

7 Growth pattern of juvenile anchovy and sardine in different areas/environments

During the exchange program and during the workshop the analysis of juveniles' otolith images permitted to highlight differences in the readability of the otolith collections coming from the participating countries/areas. Different number of checks (false annulus) were observed and a generalized more complexity in the interpretation and age assignment mainly in Mediterranean areas. Such general considerations induced the workgroup to evaluate whenever differences in growth rates and patterns from different areas could support the sudden complexity in otolith from Mediterranean areas.

Length-at-age plots and increment width curves for each area and both species were obtained to provide more information on the otolith sets analysed during WKMIAS and to evaluate if the differences in the environments of the represented areas could be reflected by different growth patterns.

Unfortunately, in the framework of this workshop, anchovy growth patterns comparison studies among some areas cannot be performed. This is due to differences in daily increment interpretation criteria. As previously mentioned, some institutes follow the criterion suggested by Cermeño *et al.* (2008) and Morales-Nin *et al.* (2010) for this species. That is, the GBR method for ageing anchovy is employed in the Bay of Biscay, North Aegean Sea and Western Mediterranean Sea. However, in the Strait of Sicily and Adriatic Sea the IMR criterion is employed. Therefore, the observed differences should be related to the different interpretation criteria.

7.1 Collection of information on growth patterns

To provide more information on the otolith sets analysed during WKMIAS and to evaluate if differences arises in growth patterns among areas, growth patterns as indicated by age length plots and increment width were provided for each area. The data, where available, were separated for life stage both for anchovy than sardine. Unfortunately not all the life stages have been studied in all the considered areas and thus mainly juveniles were presented in this report.

Anchovy

Bay of Biscay

Anchovy larvae:

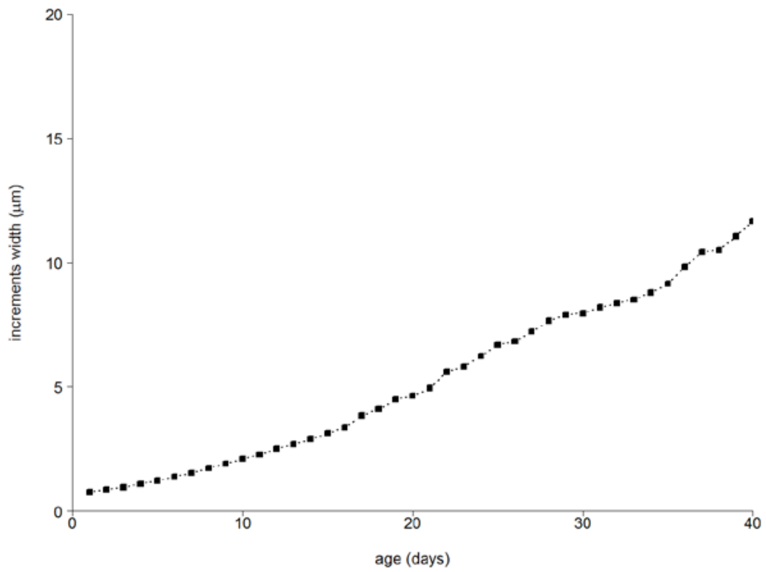


Figure 9 Average increment width at age for anchovy larvae in the Bay of Biscay.

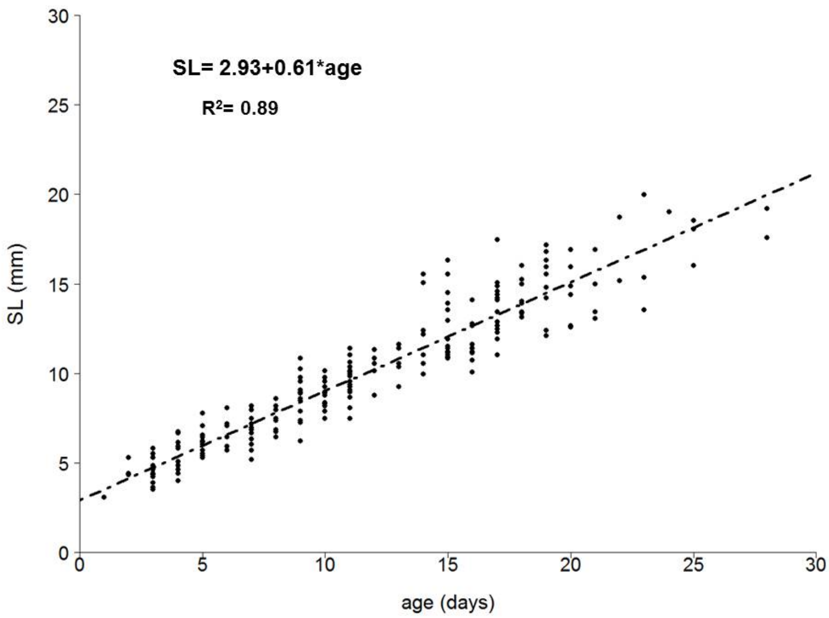


Figure 10 Relationship between standard length (SL) and age of anchovy larvae.

Anchovy juveniles:

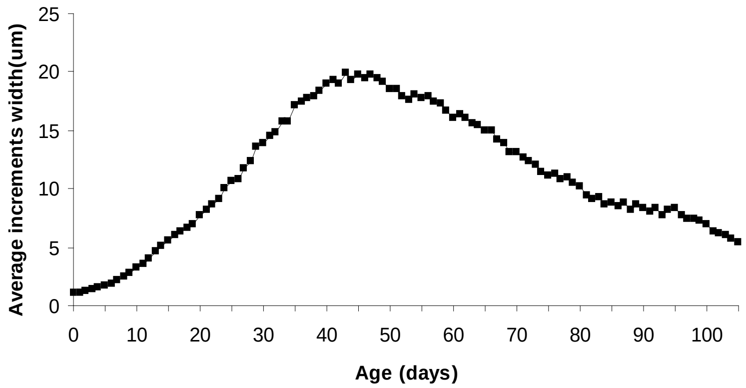


Figure 11 The width of increments pattern showed a first zone increasing from 1 to 18 µ at 45 days, while after this age the width decreases slowly to reach 5 µ at 105 days.

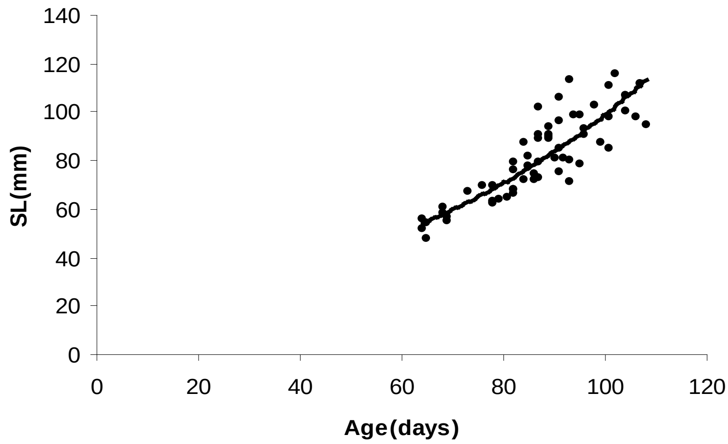


Figure 12 Age at length (standard length =SL) data for juveniles anchovy from the Bay of Biscay.

North Aegean Sea

Anchovy larvae:

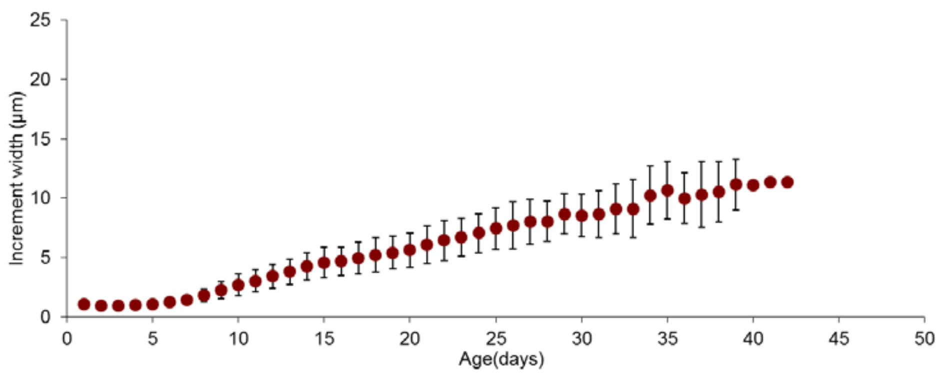


Figure 13: Mean increment width by age along with error bars (standard deviations).

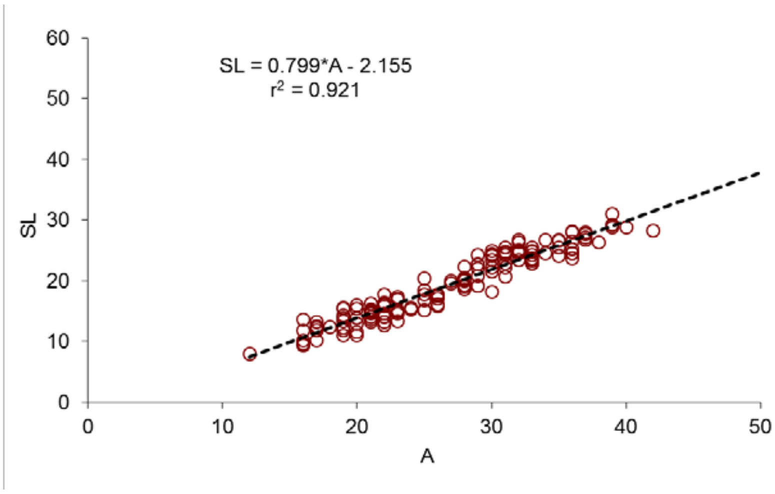


Figure 14: Relationship between standard length (SL) and age (A) for anchovy larvae.

Anchovy juveniles:

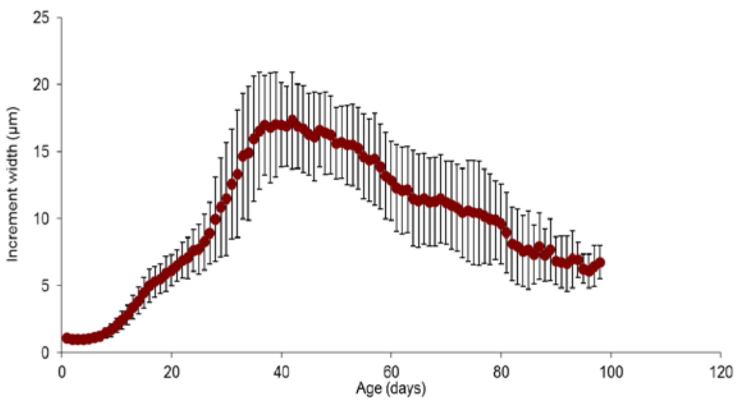


Figure 15 Mean increment width by age along with error bars (standard deviations) The width of increments pattern showed a first zone increasing from 1 to 17 µ at 40 days, while after this age the width decreases slowly to reach 7.5 µ at 100 days.

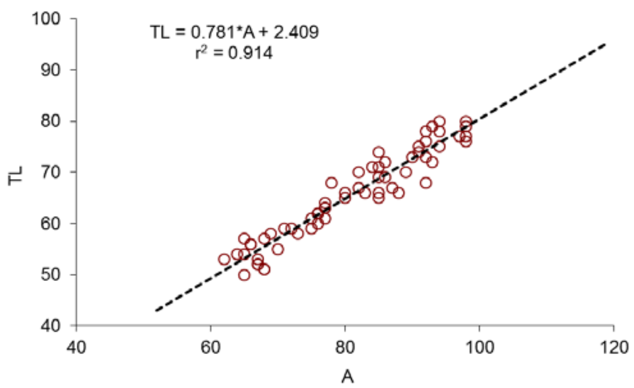


Figure 15 Relationship between total length (TL) and age (A).

Strait of Sicily

Anchovy juveniles

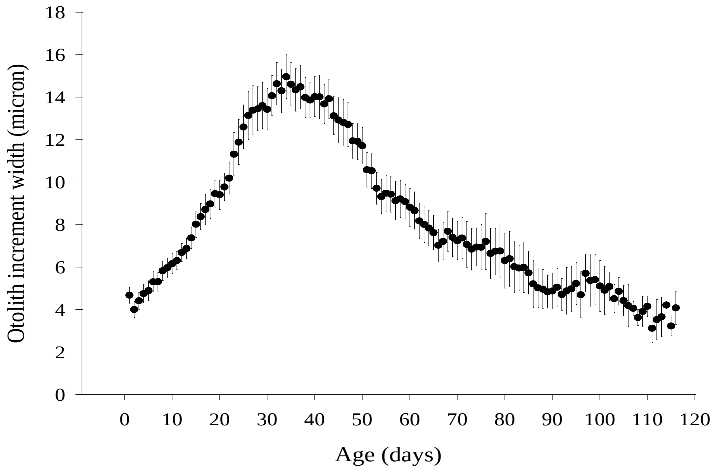


Figure 16 The width of increments pattern showed a first zone increasing from 4 to 14 μ at 35 days, while after this age the width decreases slowly to reach 3 μ at 115 days.

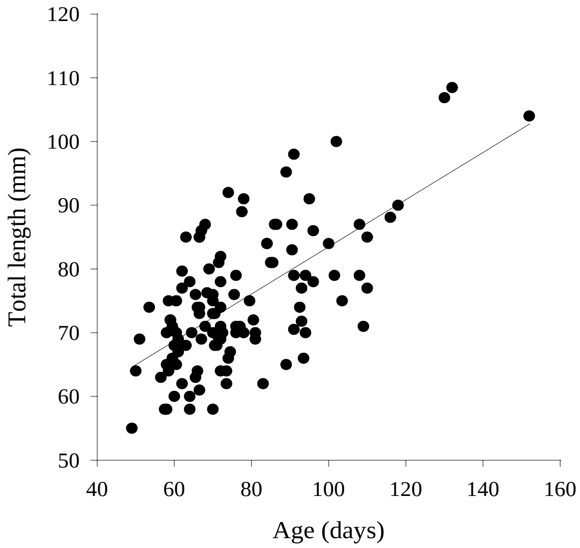


Figure 17 Age at length data for juveniles anchovy from the Strait of Sicily: Average growth rate=0.4 mm d-1 (Intercept= 46.47; Slope= 0.37; r^2 = 0.41). The data showed juveniles presence until 11 cm total length during the recruitment peak.

Adriatic Sea

Anchovy juveniles

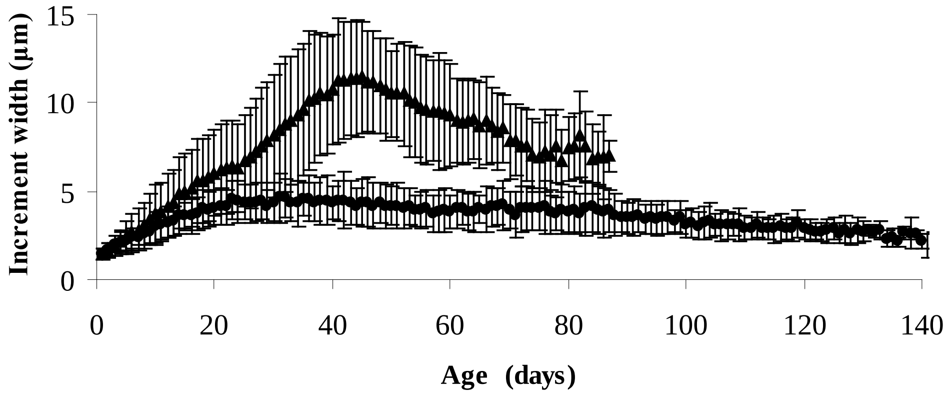


Figure 18 Otolith mean increment width vs. age (days) for anchovy individuals caught in Adriatic Sea (Po River) in November 2007 (triangle) and in February 2009 (circle).

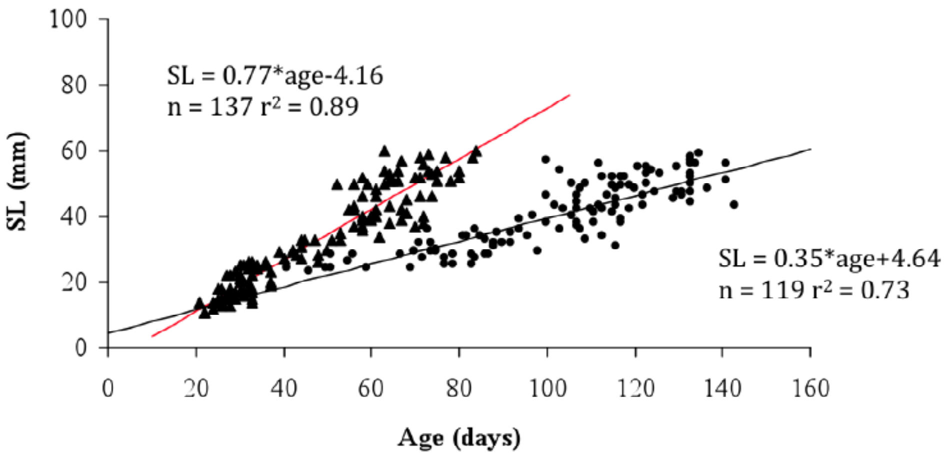


Figure 19: Age at length (standard length=SL) data for anchovy individuals caught in Adriatic Sea (Po river) in November 2007 (triangle) and in February 2009 (circle). Average growth rate=0.77 mm/day and 0.35 mm/day respectively.

South Spain (Western Mediterranean Sea)

Anchovy Larvae

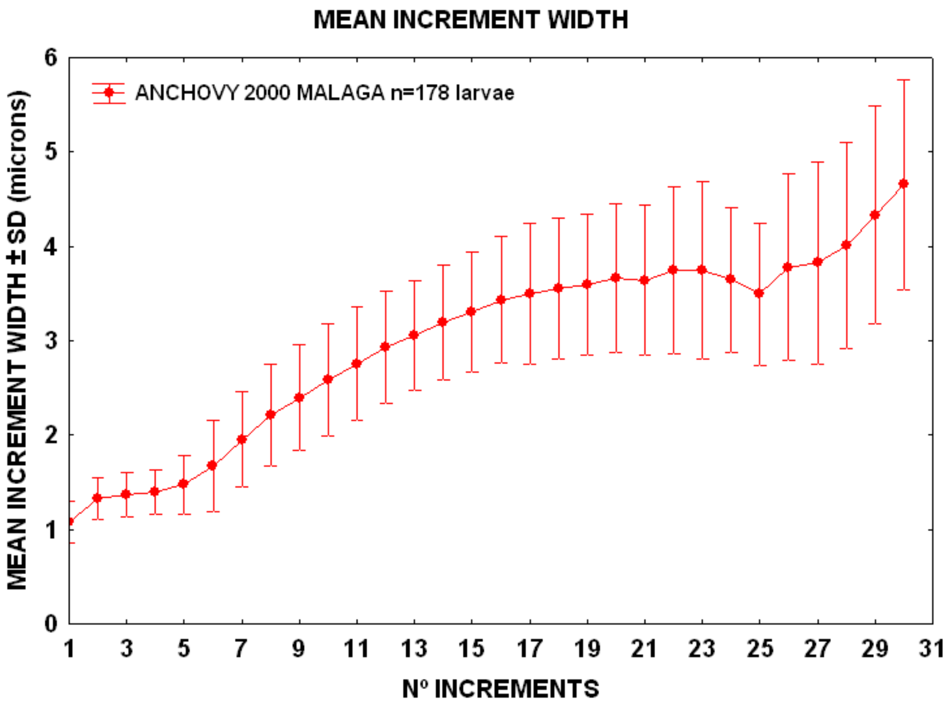


Figure 20: Anchovy larvae mean increment width by age along with error bars (standard deviations).

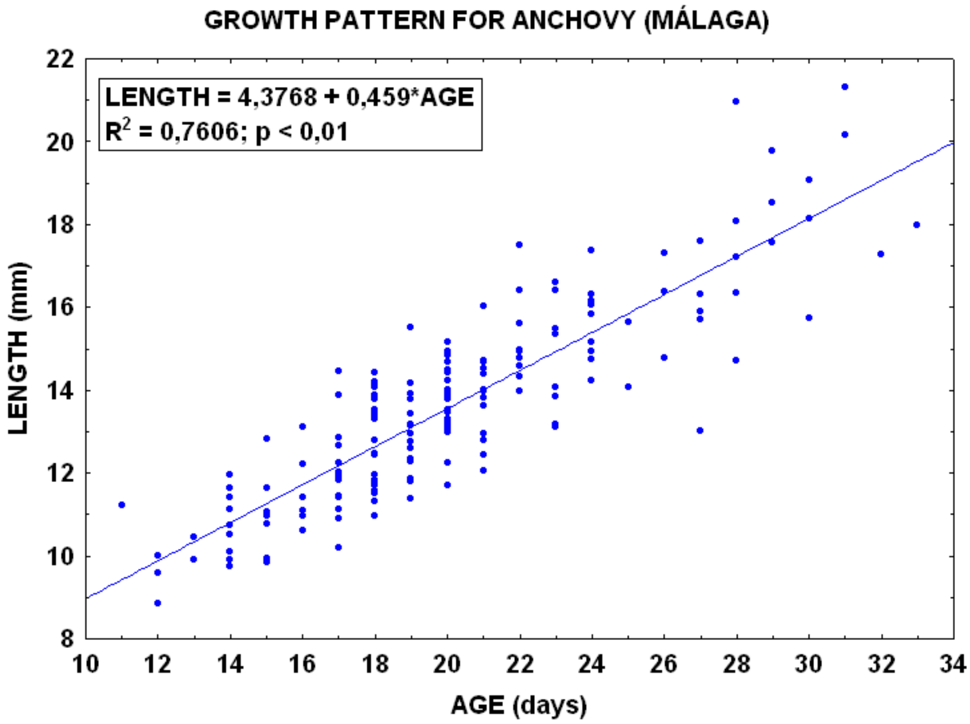


Figure 21: Relationship between standard length (SL) and age (days) for anchovy larvae.

Sardine

Bay of Biscay

Sardine larvae

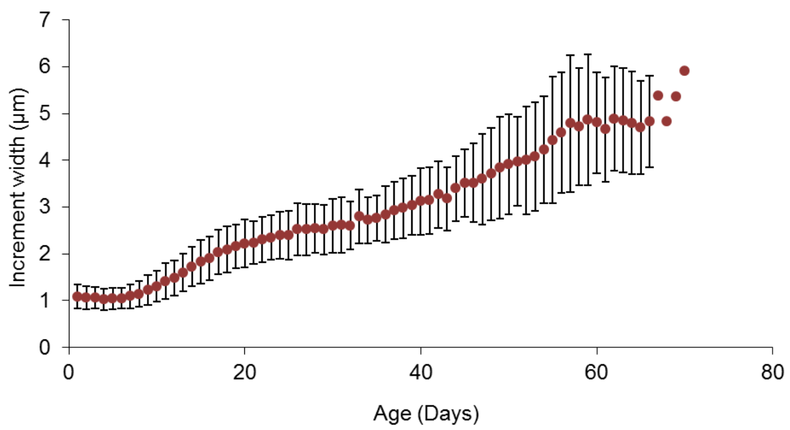


Figure 22 Mean increment width by age along with error bars (standard deviations).

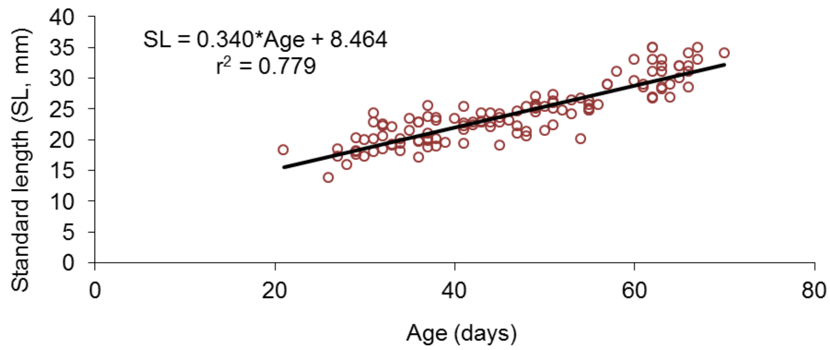


Figure 23: Relationship between standard length and age.

Sardine juveniles

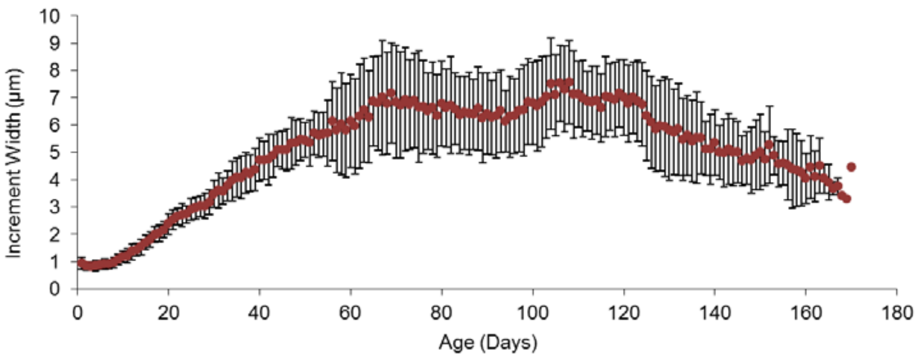


Figure 24 Mean increment width by age along with error bars (standard deviations).

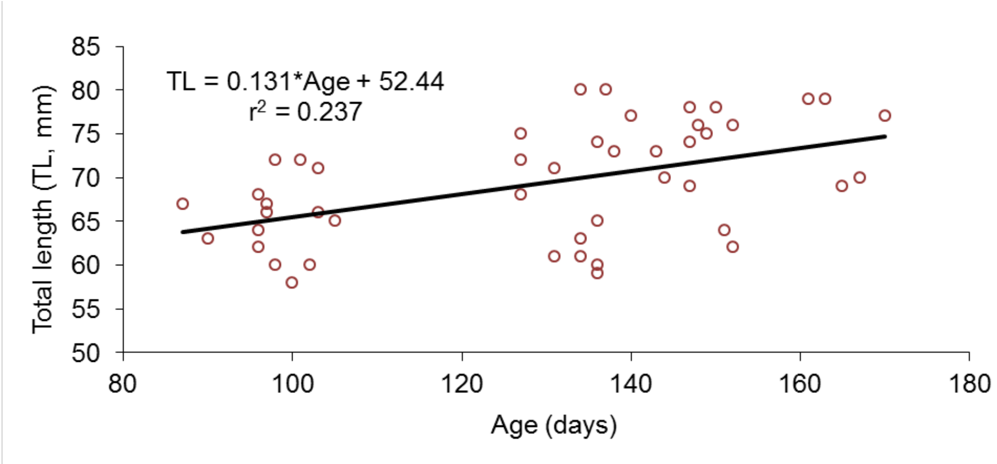


Figure 25 Relationship between total length and age.

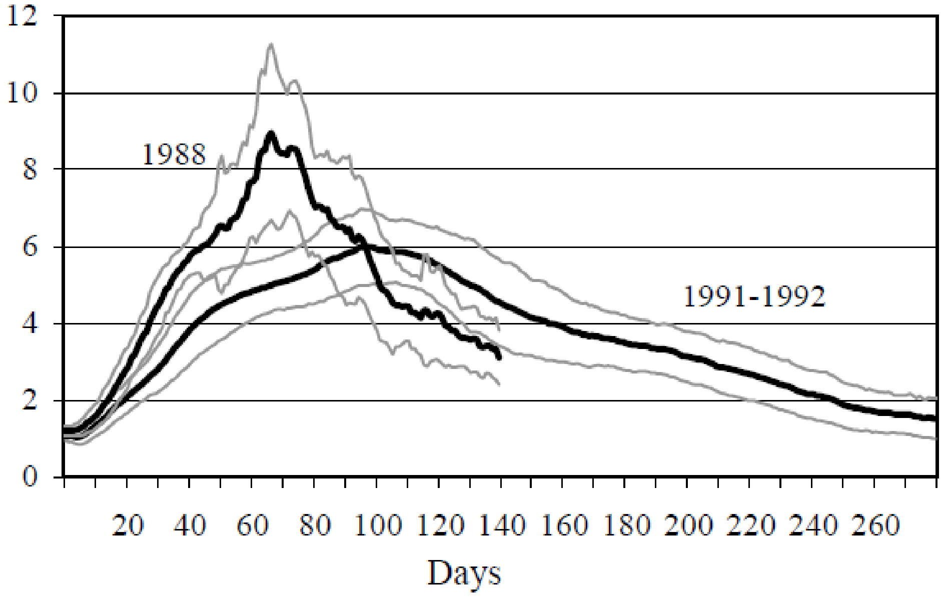


Figure 26 Otolith mean increment width vs. age (days) for individuals born in spring (1988) and winter (1991-1992).

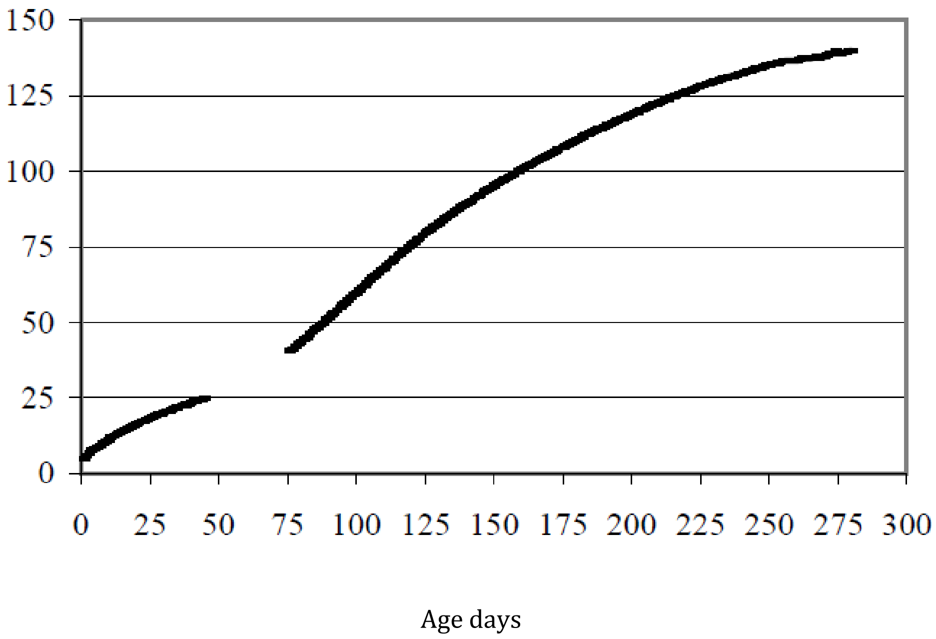


Figure 27: Back calculated ages (a) Until 45 days, size =ln(otolith radius*1.3)/0.14, n=99, r^2=0.951; b) From 75 days, size=0.22+otolith radius0.92, n=275, r^2= 0.887.

South Spain (Western Mediterranean Sea)

Sardine Larvae

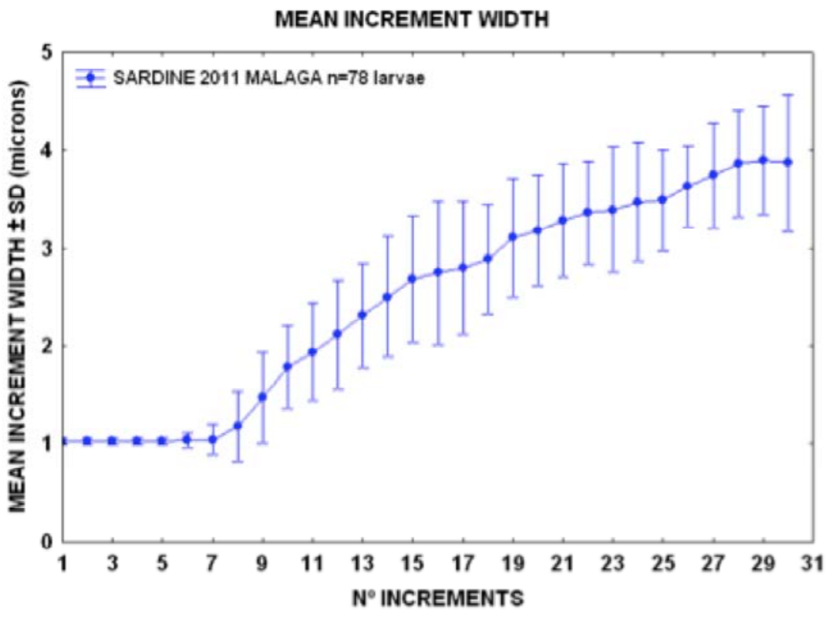


Figure 28: Mean increment width by age along with error bars (standard deviations).

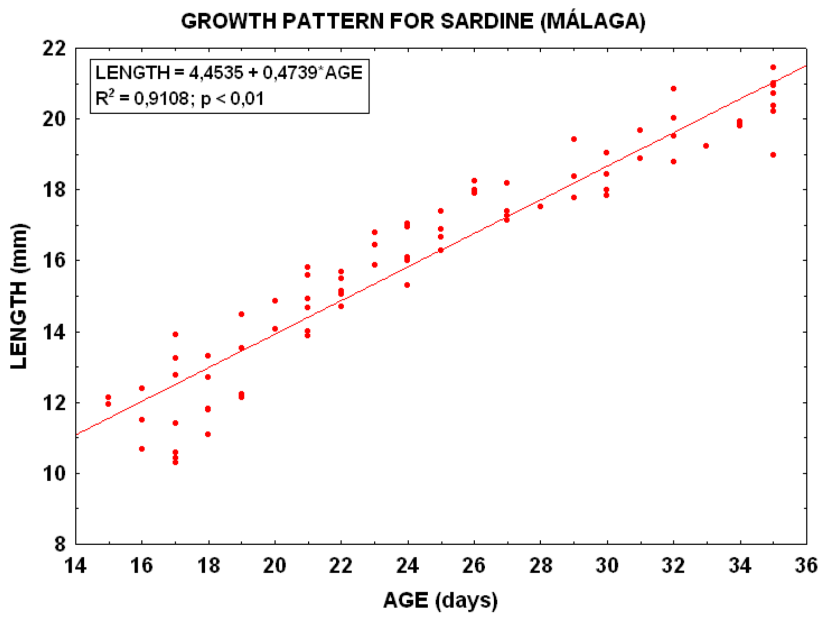


Figure 29: Relationship between standard length (SL) and age (days) for sardine larvae.

7.2 General considerations for each life stage and species

Anchovy larvae

Anchovy larvae information were available only from three areas the North Aegean Sea, the Western Mediterranean (Malaga Waters) and the Bay of Biscay.

In the North Aegean Sea the increment width increases from 3 µm at 10 days to 11 µm at 43 days, while in western Mediterranean (Malaga waters) increases from 1 µm at 8 days to 4 µm at 30 days. In the Bay of Biscay, average increment width increases from 2 µm at 10 day to 12 µm at 40 days.

In the North Aegean Sea the specimens considered were between 6-7 mm to 27-28 mm SL. Namely individuals smaller than 6-7 mm SL were 11 days, while specimens of almost 42-43 days were 27-28 mm long. In western Mediterranean Sea the specimens ranged between 10 mm to 18 mm TL. Namely larvae smaller than 10 mm TL were 12 days old, while specimens of 18 mm long were almost 30-34 days old. In the Bay of Biscay, anchovy SL ranged between 3 and 19 mm for individuals of between 1 and 28 days old.

A first raw comparison of such data displayed clear differences in growth rate along the whole considered period. The increment width in the Eastern Mediterranean Sea reached the maximum growth increments observed in Western Med (4 µm), almost 20 days before.

Age and length comparison showed that at 30 days old in West Med the average length of larvae is 18 mm, in the East Med 22 mm and 21 mm in the Bay of Biscay.

Further investigations more addressed to growth comparison among areas would be necessary to obtain statistical significance of differences.

Anchovy juveniles

As previously mentioned, in the Bay of Biscay and in the North Aegean Sea the GBR criterion has been employed for daily increment interpretation, whereas in the Strait of

Sicily and in the Adriatic Sea the IMR method has been followed. Therefore, some areas cannot be compared since different interpretation criteria have been used.

The observed increment width patterns along the first 100 days appear different among areas.

In the Bay of Biscay the width increments pattern recorded values reached 18 µm at 45 days. Similarly in the Greek waters, the growth of increment width reached the value of 17 µm (maximum width) in 38 days. In the Strait of Sicily and Adriatic Sea the maximum increment width have lower value respectively of 14 µm at 38 days old and 12 µm at 45 days. In Adriatic Sea individuals caught in February 2009 gave quite lower values with a more regular growth pattern fluctuating around 4 µm.

The decreasing trend after the peak of increment width appear similar among areas: in the Bay of Biscay the width decrease from 45 to 100 days respectively with 18 and 6-7 µm, similarly in Aegean Sea from 40 to 100 days the increment width decreases from 27 to 7-8 µm. In the Strait of Sicily the growth increment trends appears quite similar to the other areas even starting from lower width (15 instead of 17-18 µm) and reaching 5 µm at 100 days. A pattern similar to the Strait of Sicily was also observed in Adriatic Sea where the increment width decreases respectively from 12 µm (at 45 days) to 6 µm (at 90 days). For individuals caught in February 2009 the width remains quite constant around 4 µm.

In the Bay of Biscay the specimens considered were between 50 to 120 mm standard length, corresponding respectively to an average age of 65 and 110 days. On the basis of the previous information it is worth noting that in this area three months old Juveniles may reach 12 cm SL (~13 cm TL). In the Bay of Biscay, the 80 mm total length was reached at 85 days old.

In the Aegean Sea were recorded values between 50 mm (at 65 days) and 80 mm total length (at 100 days old). In this area juveniles reached 80 mm length, 15 days earlier than in the Bay of Biscay.

In the Strait of Sicily the observed size at age range was from 55 mm (50 days old) to 110 mm (~150 days). In such area anchovy juveniles of 80 mm total length are in average 95 days old. In Adriatic Sea the individuals considered size range was further reduced compared to the previous areas, with a lower starting size of 10 mm reaching 60 mm standard length (~70 mm TL). Namely individuals until 10 mm SL were younger than 20 days old and specimens of 60 mm length (sampled in November) were almost 85 days old.

Sardine larvae

In the North Aegean Sea the maximum width increments pattern recorded values reached 5 µm at 70 days, while for Western Mediterranean is 4.5 µm at 30 days.

In the North Aegean Sea the width increment increases from 1 µm at 10 days to 5 µm at 70 days, while in Western Mediterranean increases from 1 µm at 2 days to 4.5 µm at 30 days. The Western Mediterranean larvae appear with a faster increment growth reaching a value similar to the maximum attained in Aegean Sea many days before (25).

In the North Aegean Sea the specimens considered were between 10 mm to 35 mm SL respectively with ages of 20 and 70 days old. In Western Mediterranean the specimens considered were between 12 mm to 21 mm TL respectively, with ages of 15 and 35 days old. In the Aegean Sea 20 mm standard length was reached at 38 days suggesting similar growth between the two areas.

Sardine juveniles

In North Aegean Sea increments maximum value was 7 μm at 110 days; 40 days later than in the Bay of Biscay where individuals caught in spring (1998) recorded the maximum value of 9 μm at 70 days. For specimens caught in winter (1991-1992) the increments pattern recorded values reached 6 μm at 100 days.

The width increments in North Aegean Sea decreased from 7 to 3 μm respectively at 110 and 170 days old, while in the Bay of Biscay for vernal specimens the width decrease from 9 to 3 μm respectively at 70 and 140 days. For wintry individuals the width decreases from 6 to 2 μm respectively at 100 and 280 days old. This part of the increments patterns doesn't differ between the two areas.

Regarding the length-at-age data: in North Aegean Sea the individuals were between 57 and 83 mm in total length. Respectively with ages of 90 and 170 days; in the Bay of Biscay for both sampling periods (spring 1988 and winter 1991-1992) the individuals considered were between 40 mm (75 days) to 140 mm total length (280 days). Individuals smaller than few millimetres TL were almost 1 day, while specimens of 280 days were 140 mm long. In the Bay of Biscay sardine juveniles reached 83 mm standard length in 137 days but if we convert from standard to total length the size of 83 mm TL is reached many days before than in Greek waters further supporting a higher growth rate in Bay of Biscay sardine.

7.3 Concluding remarks

Even a detailed analysis to evaluate growth differences across areas is out of the main scope of the present report the visual inspection, as previously described, suggest that differences may exist in the otolith growth patterns which could be misleading in the interpretation work. In some area the laid down of ring determine a complex structure which increase the probability of an interpretation mistake.

More detailed analysis with data from several different areas is strongly recommended to address these important aspects.

However the information collected from the working group and resumed in the present section strongly suggest it necessary to carry out a specific study based on the data collected and on the literature revision. Such study would be possible thanks also to the standardization work carried out during WKMIAS.

Finally it has bearing in mind that the previous results were obtained before the standardization work carried out among participants during WKMIAS and this may affect the comparison of growth patterns from different areas, due to the variability of otolith thin sections preparation techniques and interpretation criteria adopted in different labs.

In the present report, however, were provided otolith images for each area which may permit a practical guide for readers to overcome mistakes due to structural differences in the assignment of age among areas.

8 Precision and accuracy of age estimates by micro-increment counts

Before the foreseen workshop on daily age reading, it has been considered useful to plan an exchange programme of anchovy and sardines otolith images in order to ascertain the current level of precision among Institutes and the difficulties that the daily age reading of anchovy and sardine otoliths present. To that purpose, an exchange programme of anchovy and sardine otoliths has been organized between June and September 2013 and the results (Villamor *et al.*, 2013 WD-Annex 4) were presented in the WKMIAS. During the workshop, we were identifying and discussing the growth patterns and the different criteria for assigning daily ages in each area (See Section 7.3), using images of the otoliths of the exchange and also new ones. After this exercise, each reader individually read a sample of sardine and anchovy in order to evaluate the improvement in the ageing precision by each reader. The main results are summarized below.

8.1 Material and Methods

8.1.1 Participants and qualification of readers

A total of 12 readers with different levels of experience of anchovy and sardine otolith daily reading participated in the otolith exchange, from different research institutions from France, Spain, Portugal, Italy and Greece and from the different areas concerned (Tables 8.1 and 8.2). Nine of them participated in the exchange of otoliths of anchovy and another nine in the otoliths of sardine. Five of them participated in both exchanges. Anchovy readers had more experience (only one with low level) than sardine readers (five readers with low level).

All readers who participated in the anchovy and sardine Exchange also attended the workshop, except the Ifremer reader (R1 in the exchange of the two species) and the IEO-Vigo reader (R2 in the exchange of sardine). A new reader from AZTI with high experience in the reading of anchovy participated in the workshop. Although all readers participants read the otoliths at the workshop, for the purposes of analysing only the results of the Exchange reader new participant were excludes from the analysis.

Table 8.1. Exchange /workshop ANCHOVY participants, with reader's identification (ID), their associated institution/laboratory, country, level of experience of anchovy daily ageing and areas where they have experience.

Name	Institute/Laboratory	Country	Reader ID	Live stage anchovy experience	Experience level	Stock/Area of expertise	Exchange/Worshop participant
Patrick Grellier	IFREMER/ Nantes	France	R1	Larvae&juvenile	High	Bay of Biscay	Exchange
Naroa Aldanondo	AZTI/ Pasaia	Spain	R2	Larvae&juvenile	High	Bay of Biscay	Both
Carmen Hernadez	IEO/ Santander	Spain	R3	Juvenile	High	Bay of Biscay	Both
Jose Mª Quintanilla	IEO/ Málaga	Spain	R4	Larvae	High	Western Mediterranean	Both
Salvatore Gancitano	CNR-IAMC/Sicily	Italy	R5	Juvenile	High	Strai of Sicily	Both
Fortunata Donato	CNR-ISMAR/Ancona	Italy	R6	Larvae&juvenile	High	Adriatic Sea	Both
Monica Panfili	CNR-ISMAR/Ancona	Italy	R7	Larvae&juvenile	High	Adriatic Sea	Both
Elisa Domenella	CNR-ISMAR/Ancona	Italy	R8	Larvae&juvenile	Low	Adriatic Sea	Both
Eudoxia Schismenou	HCMR/Crete	Greece	R9	Larvae&juvenile	High	North Aegean Sea	Both
Beatriz Beldarrian	AZTI/ Pasaia	Spain	R10	Larvae&juvenile	High	Bay of Biscay	Workshop

Table 8.2. Exchange /workshop SARDINE participants, with reader's identification (ID), their associated institution/laboratory, country, level of experience of anchovy daily ageing and areas where they have experience.

Name	Institute/Laboratory	Country	Reader ID	Live stage sardine experience	Experience level	Area of expertise	Exchange/Workshop participant
Patrick Grellier	IFREMER/ Nantes	France	R1	Larvae&juvenile	High in larvae, Low in juveniles	Bay of Biscay	Exchange
Eduardo López	IEO/ Vigo	Spain	R2	Juvenile	Low	Atlantic Iberian	Exchange
Andreia Silva	IPMA/Lisbon	Portugal	R3	Juvenile	Low	Atlantic Iberian	Both
Jose Mª Quintanilla	IEO/ Málaga	Spain	R4	Larvae	High	Western Mediterranean	Both
Salvatore Mangano	CNR-IAMC/Sicily	Italy	R5	Juvenile	Low	Strai of Sicily	Both
Fortunata Donato	CNR-ISMAR/Ancona	Italy	R6	Larvae&juvenile	High	Adriatic Sea	Both
Monica Panfili	CNR-ISMAR/Ancona	Italy	R7	Larvae&juvenile	High	Adriatic Sea	Both
Elisa Domenella	CNR-ISMAR/Ancona	Italy	R8	Larvae&juvenile	Low	Adriatic Sea	Both
Eudoxia Schismenou	HCMR/Crete	Greece	R9	Larvae&juvenile	High	North Aegean Sea	Both

8.1.2 Otolith images of Anchovy and Sardine collection

For the exchange we have selected images by species and sampling area, with a total of 81 images distributed as follows: for anchovy 41 images of otoliths were analysed for daily age assignment, distributed in 5 sets from different anchovy distribution areas (Figure 30 and Table 8.3); and for sardine 40 images of otoliths were analysed for daily age assignment, distributed in 5 sets from different sardine distribution areas (Figure 30 and Table 8.4). In the case of sardines, also a small otoliths collection (5) of known age (obtained from Aquaculture) was used.

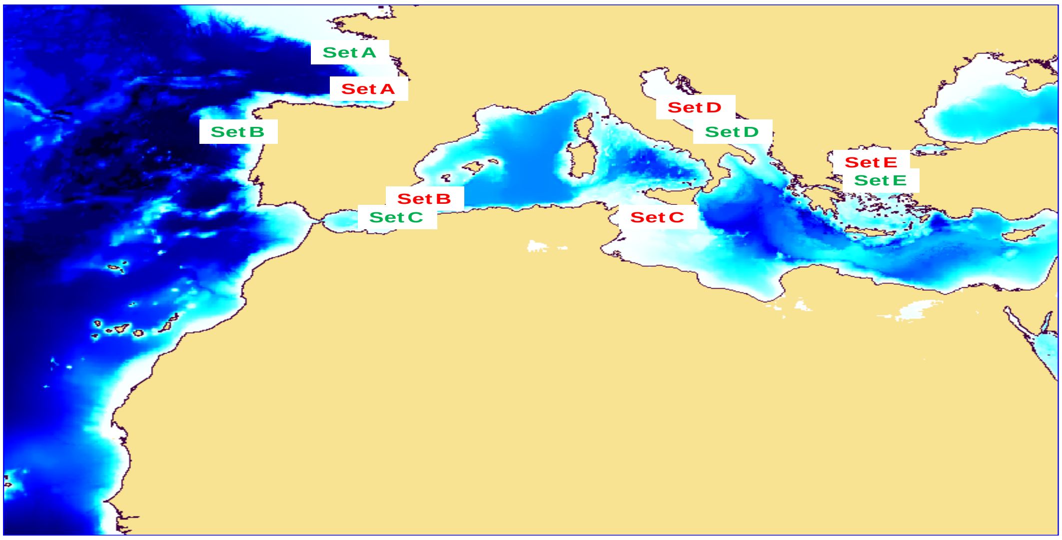


Figure 30. Collection areas of 2013 otolith image exchange sample sets of anchovy (in red) and sardine (in green)

During the workshop we used an exchange sample of each species. In the case of sardine, the sample was a juvenile from Aquaculture in the Atlantic Iberian, and the sample of anchovy corresponded to a juvenile from Adriatic Sea.

Table 8.3 Summary of ANCHOVY otolith images sets used for age reading.

	Anchovy samples					
SET- Area	Zone	Number of images	Length range SL (mm)	Year	Institute	Remarks
SET A- Bay of Biscay	ICES Division VIIIbc	10	20.4-106	2005-2007-2009	IEO Santander/AZTI	Larvae & juveniles
SET B- Western Mediterranean	Almeria Bay	5	14.7-20.7	2011-2012	IEO Malaga	Larvae
SET C- Strait of Sicily	GS16	5	56-69	2005	IAMC-CNR	Juveniles
SET D- Adriatic Sea	Manfredonia/ Ortona	11	26-63	1996-1997-2013	ISMAR-CNR	Larvae & juveniles
SET E- North Aegean Sea	North Aegean Sea	10	12.01-75	2007	HCMR	Larvae & juveniles
Total		41	12.01-106			

Table 8.4. Summary of SARDINE otolith images sets used for age reading

	Sardine samples					
SET- Area	Zone	Number of images	Length range SL	Year	Institute	Remarks
SET A- Bay of Biscay	ICES Division VIIIab	5	17.1-21.6	2012	IFREMER	Larvae
SET B- Atlantic Iberian	ICES Division IXa	10	54.2-136	04-05-08-09-10	IEO Vigo/IPMA	Juveniles (5 from culture)
SET C- Western Mediterranean	Almeria Bay	5	15-22.9	2010	IEO Malaga	Larvae
SET D- Adriatic Sea	Manfredonia/Ortona	10	14-80	1997 -2013	ISMAR-CNR	Larvae & juveniles
SET E- North Aegean Sea	North Aegean Sea	10	20.3-80	2007-2009	HCMR	Larvae & juveniles
Total		40	15-136			

8.1.3 Guideline on daily age reading

It was recommended to the Exchange that the interpretation should be done based on the guidelines established at the SARDONE project Workshop and Morales-Nin *et al.*, 2010.

The age readers deeply debated during the workshop to reach an agreement on ageing methodology. At the end of the workshop, it was not agreed interpretation criteria by areas as well as a protocol for determining the age of anchovies and sardines (see Section 7.4). Should be noted that for anchovy and sardine two different interpretation criteria are followed depends on the institute: group band reading (GBR) and individual mark reading (IMR). These were one of points most discussed during the workshop, not reaching agreement on the criteria to be followed in all areas (see Section 7.3).

8.1.4 Analysis of the daily age readings

The main lines of the work at the Workshop were as follows. The first the results and the otolith images of the 2013 Anchovy and Sardine Otolith Exchange have been discussed to improve the agreement in the ageing technique. New images of anchovy and sardine otoliths were read in live and discussed together by all readers of each species. After all these discussions on image otoliths a better agreement was expected. An individual reading of a sample should provide an estimate of the improvement in precision and of the decrease in bias. Due to the time consuming the reading of daily increments, it was decided to read just a sample of each species. First, we selected that the samples were of juveniles, because in juveniles of two species were found major discrepancies than in larvae in the exchange and also during the workshop discussions.

Sample of sardine was selected from aquaculture Atlantic Iberian set, where the accuracy in the exchange was very low. From aquaculture sample, the actual age is known and can be known well the accuracy of each reader. And in the case of anchovy, we selected a sample from Adriatic Sea, as this is where there been major discrepancies in the exchange and further discussion during the workshop. In this second reading, the readers determined the daily age for comparison with exchange results, and also, the readers measured the daily micro increments to detect whether the reading criteria had improved. Results from these last otolith samples are assumed to demonstrate the present precision in the anchovy and sardine daily age readings.

Precision was estimated using coefficient of variation (CV) of increment counting between the different readers (Chang, 1982) and also was evaluated using the average percent error (APEBF) of Beamish and Fournier (1981). Both APE and CV have been widely used as statistically measures of ageing precision in fish (Campana, 2001). As reference age, we used the mean age rather than the modal age, due to the large number of ages obtained in the daily age determination. Although the mean age estimate is not an indicator for the reliability of ageing structure, it may provide useful information regarding over- or underestimation of age by a structure irrespective of fish size class. In addition, we analysed separately the sample of aquaculture juveniles of sardine from the Atlantic Iberian set, because we knew the actual age of the specimens. We compared the increment width reported by reader, in order to know the readings interpretation from each reader in larvae and juveniles. For this, we selected image otolith readings with high level of precision in the age determination.

8.2 Results of the 2013 Otolith Image Exchange

8.2.1 Anchovy results

Table 8.2.1.1 details length and month of landing of the sets of otoliths images selected for the anchovy exchange programme by areas along with the ageing produced by each reader. The last four columns give mean daily age, standard deviation (SD) and precision of reading as the CV in relation to the average age and the APEBF. This exercise showed differences among readers and among areas. The lowest differences were found in the set of the North Aegean Sea (CV = 8.0% and APE = 7.8%) and the highest in the set of the Strait of Sicily (CV = 34.9% and APE = 23.0%). (Figure 8.2.1.1)

All images of the Bay of Biscay, Western Mediterranean and North Aegean Sea were read by all readers. However, many images of Strait of Sicily and the Adriatic Sea sets remained unread (Table 8.5). Some reasons for not reading these images were recorded by some of the readers, and were due to images calibration problems and the difficulty in identifying a growth pattern (unclear images). In these sets, the differences in the ages assigned by each reader are very large respect to the mean age (much greater than 10%). (Table 8.6 and Figure 31)

We look only at the clearer images sets (Bay of Biscay, Western Mediterranean and North Aegean Sea) to compare the results of each reader. In the Bay of Biscay set (larvae and juveniles) we found that 6 of the 9 readers have a significant agreement in the readings (the mean counts differ <10% from the mean age); R1 and R9 showed a small underestimation in their readings (between -12 and -15% relative to the mean) and R5 showed clear differences in the age interpretation criteria, over all in the older ages. Overestimation in fish age was observed in R5. In the Western Mediterranean Sea set (only larvae), the variability is higher among all readers. The differences are great in readers R5, R8 and R9, showing an overestimation in the readings of R5 and R8, and an underestimate in the reader R8. In North Aegean Sea set (larvae and juveniles), the differences are very small in the readings of all readers, showing a difference of less than 7% in 8 readers, resulting in a very high agreement among all readers in this area. The R5 has a difference of 13%, showing overestimation of younger ages (larvae). (Table 8.2.1.2 and Figure 32)

Table 8.5 ANCHOVY Otolith SET (WKMIAS 2013_Otolith Exchange)

Bay of Biscay

Sample		Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2005	m41_2	1	-	20.4	8	35	28	29	36	41	36	38	41	25	34	5.7	17%	13.5%
2005	m83_10	2	-	25.2	8	29	37	38	31	44	32	35	34	29	34	4.9	14%	10.9%
2005	m81_5	3	-	28.3	8	36	42	45	46	67	43	45	47	43	46	8.5	18%	10.6%
2005	m83_18	4	-	30.6	8	36	46	43	44	44	40	41	44	33	41	4.3	10%	8.0%
2005	m81_2	5	-	34.7	8	46	49	48	54	63	48	53	50	40	50	6.3	13%	8.7%
2007	P07_15_18	6	121	106.0	9	104	116	117	111	139	109	123	127	109	117	10.9	9%	7.0%
2009	P09_09_43	7	57	50.0	9	56	62	61	60	89	57	62	61	57	63	10.1	16%	9.3%
2009	P09_17_68	8	74	62.0	9	74	75	82	76	112	72	89	78	66	80	13.5	17%	11.5%
2009	D09_94_05	9	100	86.0	10	71	77	82	76	114	73	88	87	70	82	13.6	17%	11.6%
2009	D09_94_31	10	91.3	78.0	10	68	80	83	78	128	77	79	74	71	82	17.9	22%	12.7%
					Total read	10	10	10	10	10	10	10	10	10			15.3%	10.4%
					Total NOT read	0	0	0	0	0	0	0	0	0				

Western Mediterranean

Sample		Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2011	OTO 362-TF0811	1	-	19.9	8	29	30	26	28	37	35	31	34	23	30	4.5	15%	11.5%
2012	OTO 118a-TF0712	2	-	13.3	7	18	20	21	22	27	22	20	26	14	21	3.9	19%	13.2%
2012	OTO 118b-TF0712	3	-	13.3	7	20	20	19	21	25	21	20	24	16	21	2.6	12%	8.7%
2011	OTO 226 - TF0712	4	-	14.7	7	23	19	18	25	36	28	21	31	18	24	6.3	26%	20.7%
2012	OTO 96 - TF0712	5	-	20.7	7	38	32	32	31	45	32	30	44	22	34	7.2	21%	16.3%
					Total read	5	5	5	5	5	5	5	5	5			18.6%	14.1%
					Total NOT read	0	0	0	0	0	0	0	0	0				

Strait of Sicily

Sample		Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2005	5-22	1	68	58.0	10	72	-	-	61	128	66	66	74	89	79	23.2	29%	20.9%
2005	5-30	2	81	69.0	10	108	-	-	92	164	95	95	92	83	104	27.4	26%	17.5%
2005	5-32	3	67	56.0	10	71	-	-	63	176	77	77	69	73	87	39.8	46%	29.6%
2005	5-47	4	69	58.0	10	66	-	-	76	147	-	58	82	71	83	32.3	39%	25.5%
2005	5-50	5	70	59.0	10	64	-	-	75	145	80	67	80	68	83	28.2	34%	21.6%
					Total read	5	0	0	5	5	4	5	5	5			34.9%	23.0%
					Total NOT read	0	5	5	0	0	1	0	0	0				

Adriatic Sea

Sample		Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
1996	E149	1	55		3	65	-	-	69	-	86	85	86	60	75	11.9	16%	14.0%
1996	E481	2	26		9	36	30	22	37	39	41	41	44	18	34	9.0	26%	21.2%
1996	E666	3	46	39.0	12	65	64	69	70	74	83	78	74	48	69	10.1	14%	10.2%
1996	E685	4	35		12	54	48	53	53	55	58	59	68	48	55	6.1	11%	7.9%
1997	E696	5	75	63.0	1	83	-	-	80	-	118	128	111	81	100	21.3	21%	18.8%
1997	E807	6	66		2	85	-	-	89	-	98	104	103	67	91	14.0	15%	11.7%
2013	Ma 5	7	29	26.0	3	53	-	-	-	94	66	53	75	37	63	19.9	32%	24.3%
2013	Ma 17	8	32	28.0	3	-	36	-	-	67	73	77	73	33	60	19.9	33%	28.2%
2013	Ma 28	9	35	30.0	3	64	36	-	50	122	75	62	79	38	66	27.6	42%	29.9%
2013	Ma 57	10	40	34.0	3	89	-	-	72	125	117	80	119	48	93	28.7	31%	25.4%
2013	Ma 129	11	58	50.0	3	117	-	-	92	135	113	115	127	66	109	23.3	21%	15.8%
					Total read	10	5	3	9	8	11	11	11	11			24.0%	18.9%
					Total NOT read	1	6	8	2	3	0	0	0	0				

North Aegean Sea

Sample		Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2007	Ee_J_1-3	1	71	-	12	78	93	90	80	91	87	92	88	91	88	5.3	6%	4.6%
2007	Ee_J_1-23	2	53	-	12	70	81	81	76	77	78	76	77	72	76	3.6	5%	3.4%
2007	Ee_J_1-30	3	67	-	12	77	85	88	78	92	84	92	87	79	85	5.7	7%	5.4%
2007	Ee_J_1-32	4	59	-	12	71	81	79	73	82	75	83	82	76	78	4.3	6%	4.8%
2007	Ee_J_2-21	5	75	-	12	-	95	100	90	105	112	101	115	92	101	8.9	9%	6.9%
2007	Ee_L_11-1	6	-	30.5	7	32	33	33	33	39	33	35	34	37	34	2.3	7%	5.3%
2007	Ee_L_15-12	7	-	22.3	7	35	31	-	27	33	30	31	32	29	31	2.4	8%	5.5%
2007	Ee_L_S3-18	8	-	12.6	7	21	17	17	18	25	24	17	20	16	19	3.3	17%	13.7%
2007	Ee_L_S3-19	9	-	12.0	7	19	17	14	16	21	20	17	18	16	18	2.2	12%	9.8%
2007	Ee_L_S4-5	10	-	17.5	7	19	21	20	21	30	22	22	24	22	22	3.2	14%	8.9%
					Total read	9	10	9	10	10	10	10	10	10			9.0%	7.8%
					Total NOT read	1	0	1	0	0	0	0	0	0				

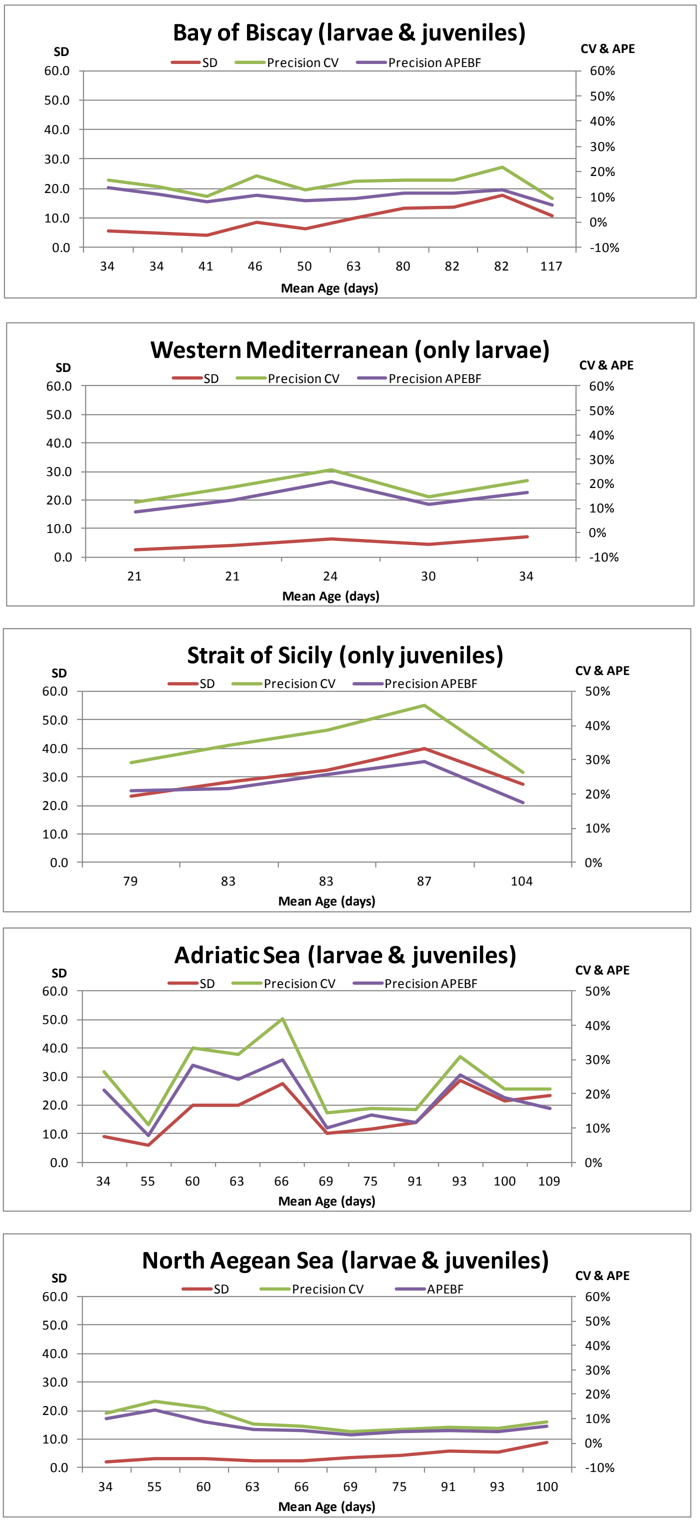


Figure 31 ANCHOVY: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against MEAN age, by sampling area.

Table 8.6 ANCHOVY: Age differences (number of days and percentages) from the mean age by sampling area and reader.

Bay of Biscay

Mean age	IFREMER R1		AZTI R2		IEO-ST R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
34	1	2%	-6	-18%	-5	-15%	2	5%	7	20%	2	5%	4	11%	6	18%	-9	-27%
34	-5	-15%	3	8%	4	11%	-3	-10%	10	28%	-2	-7%	1	2%	-1	-2%	-5	-15%
41	-5	-13%	5	12%	2	4%	3	7%	3	7%	-1	-3%	0	-1%	3	7%	-8	-20%
46	-10	-22%	-4	-9%	-1	-2%	0	0%	21	46%	-3	-7%	-1	-2%	1	2%	-3	-7%
50	-4	-8%	-1	-2%	-2	-4%	4	8%	13	26%	-2	-4%	3	6%	0	0%	-10	-20%
63	-7	-11%	-1	-1%	-2	-3%	-3	-4%	26	42%	-6	-9%	-1	-1%	-2	-3%	-6	-9%
80	-6	-8%	-5	-7%	2	2%	-4	-6%	32	39%	-8	-10%	9	11%	-2	-3%	-14	-18%
82	-11	-13%	-5	-6%	0	0%	-6	-7%	32	39%	-9	-11%	6	7%	5	6%	-12	-15%
82	-14	-17%	-2	-2%	1	1%	-4	-5%	46	56%	-5	-6%	-3	-4%	-8	-10%	-11	-13%
117	-13	-11%	-1	-1%	0	0%	-6	-5%	22	19%	-8	-7%	6	5%	9	8%	-8	-7%
Mean days	**-8**	**-12%**	**-2**	**-3%**	**0**	**-1%**	**-2**	**-2%**	**21**	**32%**	**-4**	**-6%**	**2**	**3%**	**1**	**2%**	**-9**	**-15%**

Western Mediterranean

Mean age	IFREMER R1		AZTI R2		IEO-ST R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
21	-1	-3%	-1	-3%	-2	-8%	0	2%	4	21%	0	2%	-1	-3%	3	14%	-5	-22%
21	-3	-15%	-1	-5%	0	-1%	1	4%	6	28%	1	4%	-1	-5%	5	23%	-7	-34%
24	-1	-5%	-5	-22%	-6	-26%	1	3%	12	48%	4	15%	-3	-14%	7	27%	-6	-26%
30	-1	-4%	0	-1%	-4	-14%	-2	-8%	7	22%	5	15%	1	2%	4	12%	-7	-24%
34	4	12%	-2	-6%	-2	-6%	-3	-9%	11	32%	-2	-6%	-4	-12%	10	29%	-12	-35%
Mean days	**0**	**-3%**	**-2**	**-7%**	**-3**	**-11%**	**-1**	**-2%**	**8**	**30%**	**2**	**6%**	**-2**	**-6%**	**6**	**21%**	**-7**	**-28%**

Strait of Sicily

Mean age	IFREMER R1		AZTI R2		IEO-ST R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
79	-7	-9%	-	-	-	-	-18	-23%	49	61%	-13	-17%	-13	-17%	-5	-7%	10	12%
83	-19	-23%	-	-	-	-	-8	-9%	62	75%	-3	-3%	-16	-19%	-3	-4%	-15	-18%
83	-17	-21%	-	-	-	-	-7	-9%	64	77%	-	-	-25	-30%	-2	-2%	-12	-15%
87	-16	-18%	-	-	-	-	-24	-27%	90	103%	-10	-11%	-10	-11%	-18	-21%	-14	-16%
104	4	4%	-	-	-	-	-12	-12%	60	57%	-9	-9%	-9	-9%	-12	-12%	-21	-20%
Mean days	**-11**	**-13%**	**-**	**-**	**-**	**-**	**-14**	**-16%**	**65**	**75%**	**-9**	**-10%**	**-15**	**-17%**	**-8**	**-9%**	**-10**	**-11%**

Adriatic Sea

Mean age	IFREMER R1		AZTI R2		IEO-ST R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
34	2	5%	-4	-12%	-12	-36%	3	8%	5	14%	7	20%	7	20%	10	29%	-16	-47%
55	-1	-2%	-7	-13%	-2	-4%	-2	-4%	0	0%	3	5%	4	7%	13	23%	-7	-13%
60	-	-	-24	-	-	-	-	-	7	-	13	22%	17	29%	13	22%	-27	-45%
63	-10	-16%	-	-	-	-	-	-	31	-	3	5%	-10	-16%	12	19%	-26	-41%
66	-2	-3%	-30	-45%	-	-	-16	-24%	56	86%	9	14%	-4	-6%	13	20%	-28	-42%
69	-4	-6%	-5	-8%	0	-1%	1	1%	5	7%	14	20%	9	12%	5	7%	-21	-31%
75	-10	-14%	-	-	-	-	-6	-8%	-	-	11	14%	10	13%	11	14%	-15	-20%
91	-6	-7%	-	-	-	-	-2	-2%	-	-	7	8%	13	14%	12	13%	-24	-26%
93	-4	-4%	-	-	-	-	-21	-22%	32	35%	24	26%	-13	-14%	26	28%	-45	-48%
100	-17	-17%	-	-	-	-	-20	-20%	-	-	18	18%	28	28%	11	11%	-19	-19%
109	8	7%	-	-	-	-	-17	-16%	26	24%	4	3%	6	5%	18	16%	-43	-40%
Mean days	**-5**	**-6%**	**-14**	**-20%**	**-5**	**-13%**	**-9**	**-10%**	**20**	**27%**	**10**	**14%**	**6**	**8%**	**13**	**18%**	**-25**	**-34%**

North Aegean Sea

Mean age	IFREMER R1		AZTI R2		IEO-ST R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
18	1	8%	-1	-3%	-4	-20%	-2	-9%	3	20%	2	14%	-1	-3%	0	3%	-2	-9%
19	2	8%	-2	-12%	-2	-12%	-1	-7%	6	29%	5	24%	-2	-12%	0	1%	-3	-17%
22	-3	-15%	-1	-6%	-2	-10%	-1	-6%	8	35%	0	-1%	0	-1%	1	5%	0	-1%
31	4	13%	0	0%	-	-	-4	-13%	2	7%	-1	-3%	0	0%	1	2%	-2	-6%
34	-2	-7%	-1	-4%	-1	-4%	-1	-4%	5	14%	-1	-4%	1	2%	-1	-2%	3	8%
76	-6	-8%	5	6%	5	6%	0	-1%	1	1%	2	2%	0	-1%	1	1%	-4	-6%
78	-7	-9%	3	4%	1	1%	-5	-6%	4	5%	-3	-4%	5	6%	4	5%	-2	-2%
85	-8	-9%	0	0%	3	4%	-7	-8%	7	9%	-1	-1%	7	9%	2	2%	-6	-7%
88	-10	-11%	5	6%	2	3%	-8	-9%	3	4%	-1	-1%	4	5%	0	0%	3	4%
101	-	-	-6	-6%	-1	-1%	-11	-11%	4	4%	11	11%	0	0%	13	13%	-9	-9%
Mean days	**-3**	**-3%**	**0**	**-1%**	**0**	**-4%**	**-4**	**-7%**	**4**	**13%**	**1**	**4%**	**1**	**0%**	**2**	**3%**	**-2**	**-5%**

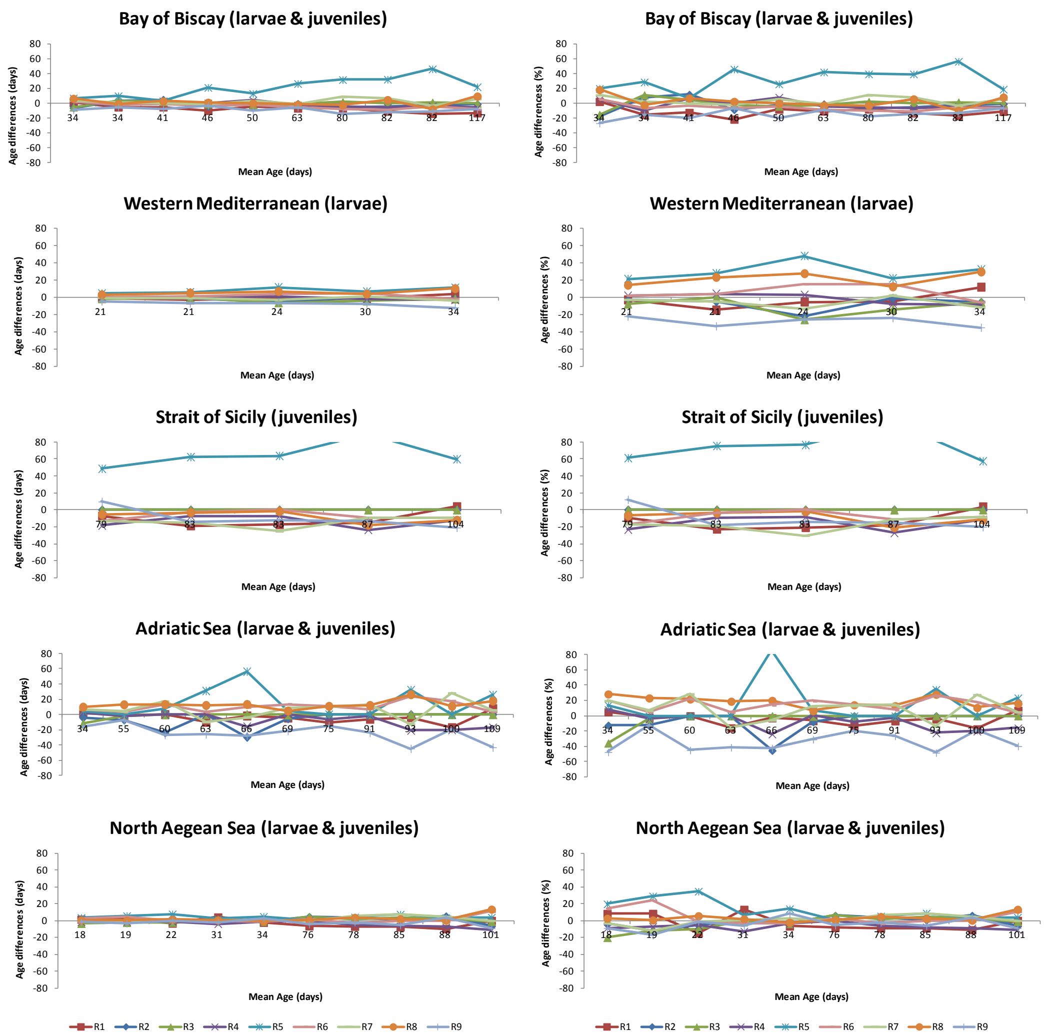


Figure 32 ANCHOVY: Age differences in days (left) and percentage (right) from the mean age by sampling area and reader.

We compared the increment width reported by anchovy reader R2, R3, R4, R5, R7 and R9 (the other readers not recorded the increments width), in order to know the readings interpretation from each reader in larvae and juveniles. For this, we selected image otolith readings with high level of precision in the age determination in the Bay of Biscay (larvae and juvenile), Western Mediterranean (larvae) and Northwest Aegean (larvae and juvenile) sets. (Figures 33 to 35)

In the Bay of Biscay, all readers appeared to apply the same reading criteria, except the reader R9 in larvae and the reader R5 in juveniles. R9 overestimated the increments width (thus underestimating the age) in the sample of larvae (code m83-18; SL= 30.6 mm; Ageing precision= 10%CV and 8%APE); R5 perhaps have applied the IMR criteria (individual increments) up to 66 days, and from there applied the GBR criteria (group bands) in the sample of juveniles (codePO7_15_18; SL= 106.0 mm; Ageing precision= 9%CV and 7%APE), overestimating the age (Figure 33).

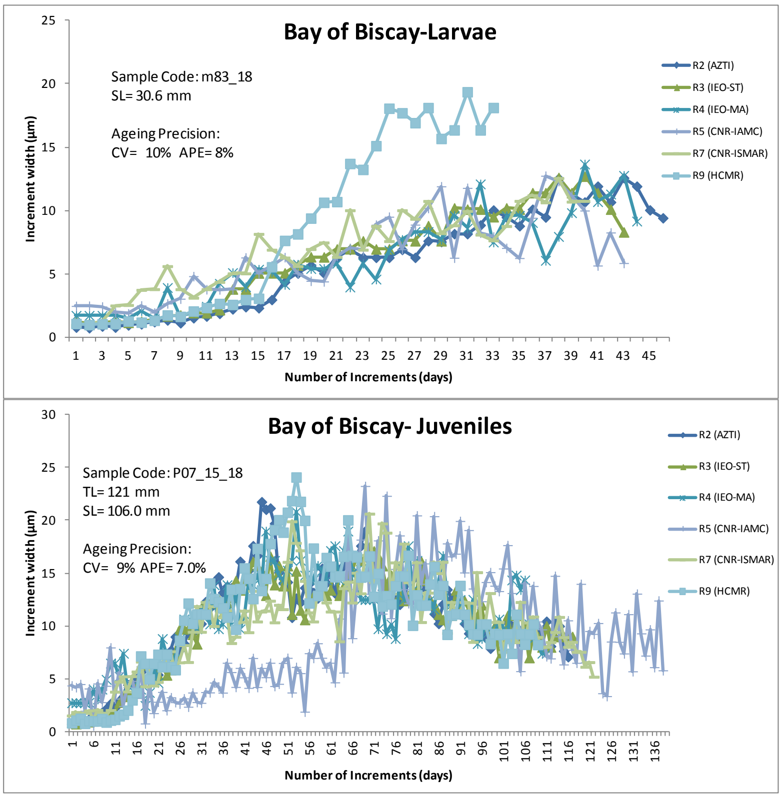


Figure 33. ANCHOVY. Comparison of the increments width results (by reader) from larvae (top panel) and from juveniles (bottom panel) of Bay of Biscay. The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

In the sample of the Western Mediterranean (Code: OTO118b-TF0712; SL= 13.3 mm; Ageing precision= 12% CV and 8.7%APE), there was greater variability of the increments width of larvae among readers. Again, the R9 overestimated the increments width underestimating the age of larvae. (Figure 34).

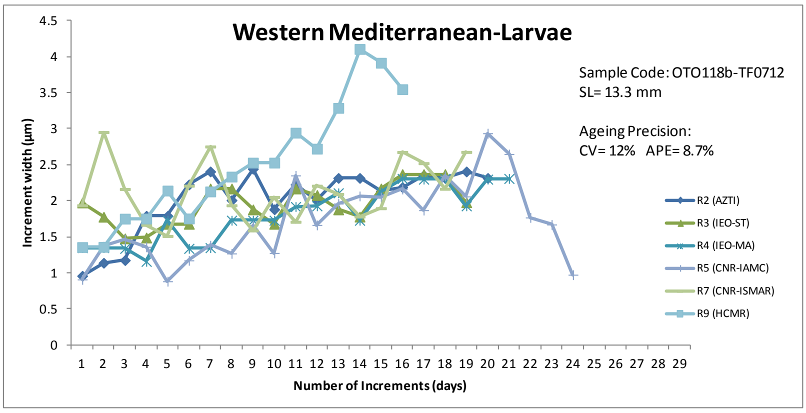


Figure 34 ANCHOVY. Comparison of the increments width results (by reader) from larvae of the Western Mediterranean. The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

In the samples of the North Aegean Sea, high increment width variability was observed in larvae (Code sample: Ee_L_11-1; SL= 30.5 mm; Ageing precision= 7% CV and 5.3%APE) among all readers, although they appeared to use the same ageing criteria. The same results and argumentations can be made for juvenile samples (Code sample: Ee_J_1-3; SL= 71 mm; Ageing precision=

6% CV and 4%APE). The largest width increments reported by the reader R5 probably could be explained more by a calibration problem than to the real assignment of increment (Figure 35).

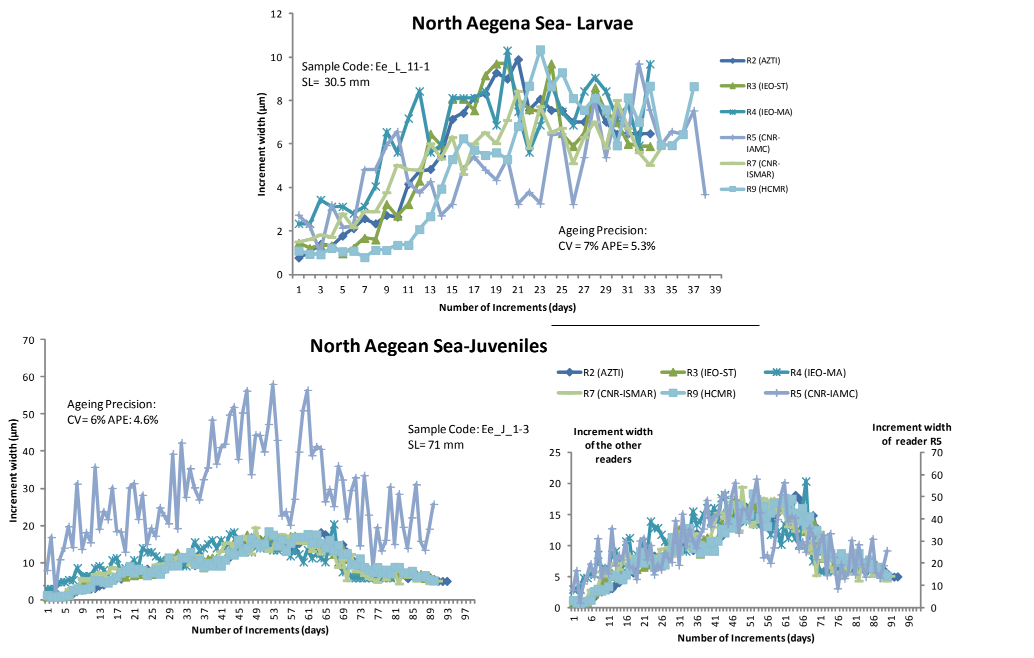


Figure 35. ANCHOVY. Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel) of the North Aegean Sea. The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

8.2.2 Sardine results

Table 8.2.2.1 details length and month of landing of the sets of otoliths images selected for the sardine exchange programme by areas along with the ageing produced by each reader. The last four columns give mean daily age, standard deviation (SD) and precision of reading as the CV in relation to the average age and the APE_{BF}.

This exchange showed differences among readers, particularly in older ages. The lowest differences were found in the set of the North Aegean Sea (CV = 8.4% and APE = 7.4%) and the highest in the set of Atlantic Iberian (CV = 18% and APE = 13.5%; Figure 36). The differences were lower in the sardine compared to anchovy in all areas, except in the North Aegean Sea. In this area, the differences are similar to those of anchovy, being the area with the best ageing precision in the two species.

All images of all areas were read by all readers, except a reader who did not read half of the images of the Adriatic Sea set (Table 8.7). In general, in larvae the age differences respect to the mean age is less than 10% for almost all readers and in all areas, except in the sample from the Bay of Biscay, where the ageing precision is lower. In this area, the reader R1 has a difference of 17%, overestimating at young ages (larvae) and readers R4 and R8 underestimating them (-17% and -12% respectively). In the Western Mediterranean set, all readers have a difference of less than 9% from the mean age of larvae, but the reader R5 overestimating the age of the larvae (an average difference of 20%). In the case of juveniles the differences are higher (greater than 30% in some cases) among readers and areas, and especially in the set of Atlantic Iberian (Table 8.8 and Figure 37).

Table 8.7 SARDINE Otolith SET (WKMIAS 2013_Otolith Exchange)

Bay of Biscay

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IFREMER R1	IEO-VI R2	IPMA R3	IEO-MA R4	CNR-IAMC R5	CNR-ISMAR R6	CNR-ISMAR R7	CNR-ISMAR R8	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
2012	St 483 - 07	1	20.45	19.5	4	26	23	25	19	18	23	27	22	26	23	3.2	14%	10.6%
2012	St 483 - 17	2	19.85	18.2	4	27	20	27	17	20	22	20	19	23	22	3.5	16%	12.6%
2012	St 533 - 11	3	21.06	19.7	5	25	22	21	18	23	20	22	22	22	22	1.9	9%	6.2%
2012	St 533 - 14	4	23.18	21.7	5	31	27	28	24	25	17	25	16	28	25	5.0	20%	15.1%
2012	St 551 - 14	5	18.79	17.1	5	23	23	21	16	27	23	22	20	23	22	3.0	13%	9.1%
					Total read	5	5	5	5	5	5	5	5	5			14.5%	10.7%
					Total NOT read	0	0	0	0	0	0	0	0	0				

Atlantic Iberian

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IFREMER R1	IEO-VI R2	IPMA R3	IEO-MA R4	CNR-IAMC R5	CNR-ISMAR R6	CNR-ISMAR R7	CNR-ISMAR R8	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	79	16.0	20%	15.0%
2010	sc_180810_6_1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	89	12.7	14%	9.4%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	100	13.5	14%	11.3%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	113	9.8	9%	6.8%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	130	14.5	11%	8.9%
2008	21_10_08_33_200x	6	160	136.0	10	185	278	257	214	162	252	251	293	123	224	56.9	25%	21.0%
2009	16_04_09_01_200x	7	144	123.0	4	217	222	267	255	151	237	175	265	150	215	46.4	22%	17.6%
2004	20_07_04_71_200x	8	138	117.0	7	136	214	189	175	160	290	177	184	121	183	48.9	27%	17.7%
2004	27_05_04_07_200x	9	84	71.0	5	108	168	116	122	116	124	120	119	116	123	17.4	14%	8.2%
2005	10_01_05_02_200x	10	99	84.0	1	141	154	185	138	144	213	135	221	104	159	38.7	24%	19.6%
					Total read	10	10	10	10	10	9	10	10	10			18.0%	13.5%
					Total NOT read	0	0	0	0	0	1	0	0	0				

Western Mediterranean

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IFREMER R1	IEO-VI R2	IPMA R3	IEO-MA R4	CNR-IAMC R5	CNR-ISMAR R6	CNR-ISMAR R7	CNR-ISMAR R8	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
2010	OTO 18-TF1110	1	-	21.4	10	31	28	29	30	35	28	30	30	30	30	2.1	7%	4.3%
2010	OTO 563-TF1110	2	-	15.0	11	17	17	22	16	20	19	18	24	16	19	2.7	14%	11.4%
2010	OTO 497-TF1110	3	-	16.6	11	16	17	17	18	25	15	17	19	17	18	2.9	16%	10.4%
2010	OTO 492-TF1110	4	-	19.7	11	19	18	20	26	27	20	19	22	17	21	3.5	17%	13.1%
2010	OTO 385-TF1110	5	-	22.9	10	33	36	34	32	37	35	34	36	33	34	1.6	5%	3.8%
					Total read	5	5	5	5	5	5	5	5	5			11.7%	8.6%
					Total NOT read	0	0	0	0	0	0	0	0	0				

Adriatic Sea

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IFREMER R1	IEO-VI R2	IPMA R3	IEO-MA R4	CNR-IAMC R5	CNR-ISMAR R6	CNR-ISMAR R7	CNR-ISMAR R8	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
2013	Ms 72	1	36	32.0	3	51	-	59	64	63	63	72	68	46	61	8.6	14%	10.8%
2013	Ms 82	2	46	39.0	3	84	97	85	96	98	96	92	101	86	93	6.3	7%	5.8%
2013	Ms 121	3	39	34.0	3	62	64	69	65	68	69	68	67	58	66	3.7	6%	4.5%
2013	Ms 151	4	25	22.0	3	36	41	44	50	54	41	40	42	34	42	6.3	15%	10.8%
2013	Ms 174	5	15	14.0	3	18	20	19	21	19	18	19	21	19	19	1.1	6%	4.6%
2013	Ms 201	6	30	26.0	3	58	-	45	63	61	53	62	59	42	55	8.0	14%	11.8%
2013	Ms 231	7	43	37.0	3	75	-	96	106	96	107	109	111	80	98	13.6	14%	11.0%
1997	Sa 75	8	56		1	69	-	84	74	73	99	93	95	75	83	11.6	14%	12.1%
1997	Sa 287	9	80		5	121	-	130	167	80	211	203	194	154	157	45.4	29%	23.0%
1997	Sa 298	10	65		5	97	153	-	147	100	162	153	164	85	133	32.7	25%	21.8%
					Total read	10	5	9	10	10	10	10	10	10			14.3%	11.6%
					Total NOT read	0	5	1	0	0	0	0	0	0				

North Aegean Sea

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IFREMER R1	IEO-VI R2	IPMA R3	IEO-MA R4	CNR-IAMC R5	CNR-ISMAR R6	CNR-ISMAR R7	CNR-ISMAR R8	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
2007	Sp_J_5_7	1	80	-	7	100	98	124	107	107	118	128	126	134	116	13.1	11%	9.8%
2007	Sp_J_10_8	2	76	-	7	127	128	140	119	134	160	155	128	163	139	16.1	12%	9.7%
2007	Sp_J_10_9	3	59	-	7	126	158	133	117	125	134	151	129	141	135	13.1	10%	7.5%
2007	Sp_J_11_7	4	64	-	7	101	149	163	107	149	140	165	141	151	141	22.5	16%	11.7%
2007	Sp_J_12_22	5	60	-	7	108	137	136	111	134	135	148	129	147	132	14.0	11%	7.9%
2009	Sp_L_2-3	6	-	34.4	2	59	65	65	66	63	62	66	66	66	64	2.4	4%	3.0%
2009	Sp_L_2-18	7	-	35.4	2	64	62	68	61	61	55	63	61	66	62	3.7	6%	4.2%
2009	Sp_L_3-15	8	-	21.2	2	28	29	32	31	33	24	28	30	31	30	2.7	9%	6.9%
2009	Sp_L_3-17	9	-	20.3	2	28	29	25	30	28	25	29	32	30	28	2.3	8%	6.1%
2009	Sp_L_19-16	10	-	25.5	2	34	36	30	37	32	34	39	38	37	35	2.9	8%	6.7%
					Total read	10	10	10	10	10	10	10	10	10			9.4%	7.4%
					Total NOT read	0	0	0	0	0	0	0	0	0				

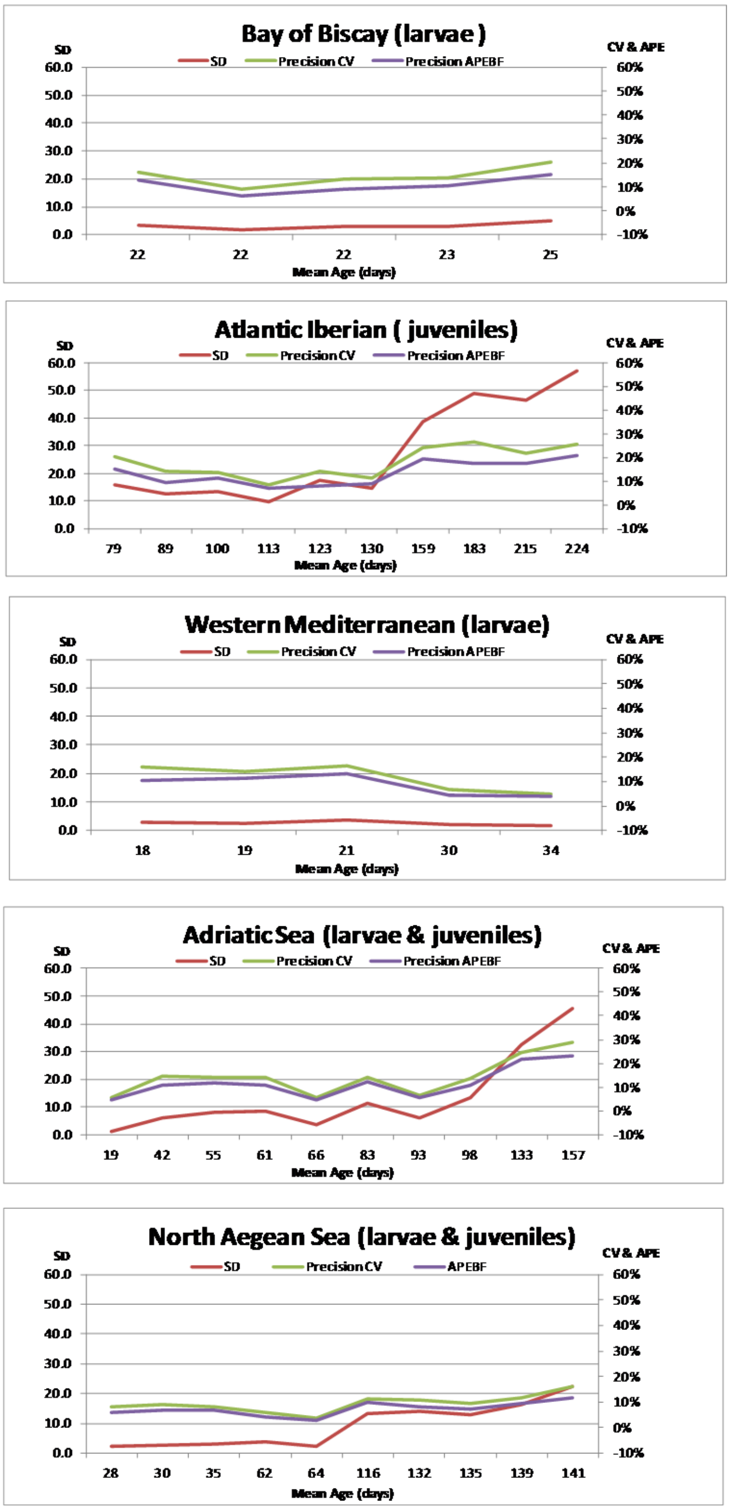


Figure 36 SARDINE: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against MEAN age, by sampling area.

Table 8.8. SARDINE: Age differences (number of days and percentages) from the mean age by sampling area and reader.

Bay of Biscay

Mean age	IFREMER R1		IEO-VI R2		IPMA R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
22	5	25%	-2	-8%	5	25%	-5	-22%	-2	-8%	0	2%	-2	-8%	-3	-12%	1	6%
22	3	15%	0	2%	-1	-3%	-4	-17%	1	6%	-2	-8%	0	2%	0	2%	0	2%
22	1	5%	1	5%	-1	-5%	-6	-27%	5	23%	1	5%	0	0%	-2	-9%	1	5%
23	3	12%	0	-1%	2	8%	-4	-18%	-5	-22%	0	-1%	4	16%	-1	-5%	3	12%
25	6	26%	2	10%	3	14%	-1	-2%	0	2%	-8	-31%	0	2%	-9	-35%	3	14%
Mean days	**4**	**17%**	**0**	**1%**	**2**	**8%**	**-4**	**-17%**	**0**	**0%**	**-2**	**-7%**	**1**	**2%**	**-3**	**-12%**	**2**	**8%**

Atlantic Iberian

Mean age	IFREMER R1		IEO-VI R2		IPMA R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
79	-10	-13%	-11	-14%	34	43%	14	17%	-3	-4%	-	-	-8	-10%	-3	-3%	-12	-15%
89	-11	-12%	-8	-8%	3	3%	32	36%	4	4%	-3	-3%	-4	-4%	-9	-10%	-5	-5%
100	-12	-12%	-11	-11%	23	23%	19	19%	8	8%	-5	-5%	-10	-10%	-4	-4%	-10	-10%
113	-13	-12%	-9	-8%	3	2%	4	3%	-7	-7%	15	13%	0	0%	14	12%	-4	-4%
123	-15	-12%	45	36%	-7	-6%	-1	-1%	-7	-6%	1	1%	-3	-3%	-4	-3%	-7	-6%
130	-14	-11%	17	13%	0	0%	20	15%	0	0%	-6	-5%	-11	-9%	16	12%	-19	-15%
159	-18	-12%	-5	-3%	26	16%	-21	-13%	-15	-10%	54	34%	-24	-15%	61	38%	-55	-35%
183	-47	-26%	31	17%	6	3%	-8	-4%	-23	-13%	107	59%	-6	-3%	1	1%	-62	-34%
215	2	1%	7	3%	52	24%	40	18%	-64	-30%	22	10%	-40	-19%	50	23%	-65	-30%
224	-39	-17%	54	24%	33	15%	-10	-4%	-62	-28%	28	13%	27	12%	69	31%	-101	-45%
Mean days	**-18**	**-13%**	**11**	**5%**	**17**	**12%**	**9**	**9%**	**-17**	**-8%**	**24**	**13%**	**-8**	**-6%**	**19**	**10%**	**-34**	**-20%**

Western Mediterranean

Mean age	IFREMER R1		IEO-VI R2		IPMA R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
18	-2	-11%	-1	-5%	-1	-5%	0	1%	7	40%	-3	-16%	-1	-5%	1	6%	-1	-5%
19	-2	-9%	-2	-9%	3	18%	-3	-15%	1	7%	0	1%	-1	-4%	5	26%	-3	-15%
21	-2	-9%	-3	-14%	-1	-4%	5	24%	6	29%	-1	-4%	-2	-9%	1	5%	-4	-19%
30	1	3%	-2	-7%	-1	-4%	0	0%	5	16%	-2	-7%	0	0%	0	0%	0	0%
34	-1	-4%	2	5%	0	-1%	-2	-7%	3	8%	1	2%	0	-1%	1	3%	-1	-4%
Mean days	**-1**	**-6%**	**-1**	**-6%**	**0**	**1%**	**0**	**1%**	**4**	**20%**	**-1**	**-5%**	**-1**	**-4%**	**2**	**8%**	**-2**	**-9%**

Adriatic Sea

Mean age	IFREMER R1		IEO-VI R2		IPMA R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
19	-1	-7%	1	3%	0	-2%	2	9%	0	-2%	-1	-7%	0	-2%	2	9%	0	-2%
42	-6	-15%	-1	-3%	2	4%	8	18%	12	27%	-1	-3%	-2	-6%	0	-1%	-8	-20%
55	3	5%	-	-	-10	-19%	8	14%	6	10%	-2	-4%	7	12%	4	7%	-13	-24%
61	-10	-16%	-	-	-2	-3%	3	5%	2	4%	2	4%	11	19%	7	12%	-15	-24%
66	-4	-5%	-2	-2%	3	5%	-1	-1%	2	4%	3	5%	2	4%	1	2%	-8	-12%
83	-14	-17%	-	-	1	2%	-9	-11%	-10	-12%	16	20%	10	12%	12	15%	-8	-9%
93	-9	-9%	4	5%	-8	-8%	3	3%	5	6%	3	3%	-1	-1%	8	9%	-7	-7%
98	-23	-23%	-	-	-2	-2%	9	9%	-2	-2%	10	10%	12	12%	14	14%	-18	-18%
133	-36	-27%	20	15%	-	-	14	11%	-33	-25%	29	22%	20	15%	31	24%	-48	-36%
157	-36	-23%	-	-	-27	-17%	10	6%	-77	-49%	54	34%	46	29%	36	23%	-3	-2%
Mean days	**-14**	**-14%**	**4**	**4%**	**-5**	**-4%**	**5**	**6%**	**-9**	**-4%**	**11**	**8%**	**10**	**9%**	**11**	**11%**	**-13**	**-15%**

North Aegean Sea

Mean age	IFREMER R1		IEO-VI R2		IPMA R3		IEO-MA R4		CNR-IAMC R5		CNR-ISMAR R6		CNR-ISMAR R7		CNR-ISMAR R8		HCMR R9	
28	0	-2%	1	2%	-3	-12%	2	5%	0	-2%	-3	-12%	1	2%	4	13%	2	5%
30	-2	-5%	-1	-2%	2	8%	1	5%	3	12%	-6	-19%	-2	-5%	0	2%	1	5%
35	-1	-3%	1	2%	-5	-15%	2	5%	-3	-9%	-1	-3%	4	11%	2	7%	2	5%
62	2	3%	0	-1%	6	9%	-1	-2%	-1	-2%	-7	-12%	1	1%	-1	-2%	4	6%
64	-5	-8%	1	1%	1	1%	2	3%	-1	-2%	-2	-3%	2	3%	2	3%	2	3%
116	-16	-14%	-18	-15%	8	7%	-9	-8%	-9	-8%	2	2%	12	11%	10	9%	18	16%
132	-24	-18%	5	4%	4	3%	-21	-16%	2	2%	3	3%	16	12%	-3	-2%	15	12%
135	-9	-7%	23	17%	-2	-1%	-18	-13%	-10	-7%	-1	-1%	16	12%	-6	-4%	6	5%
139	-12	-9%	-11	-8%	1	0%	-20	-15%	-5	-4%	21	15%	16	11%	-11	-8%	24	17%
141	-40	-28%	8	6%	22	16%	-34	-24%	8	6%	-1	0%	24	17%	0	0%	10	7%
Mean days	**-11**	**-9%**	**1**	**1%**	**3**	**2%**	**-10**	**-6%**	**-2**	**-1%**	**0**	**-3%**	**9**	**7%**	**0**	**2%**	**8**	**8%**

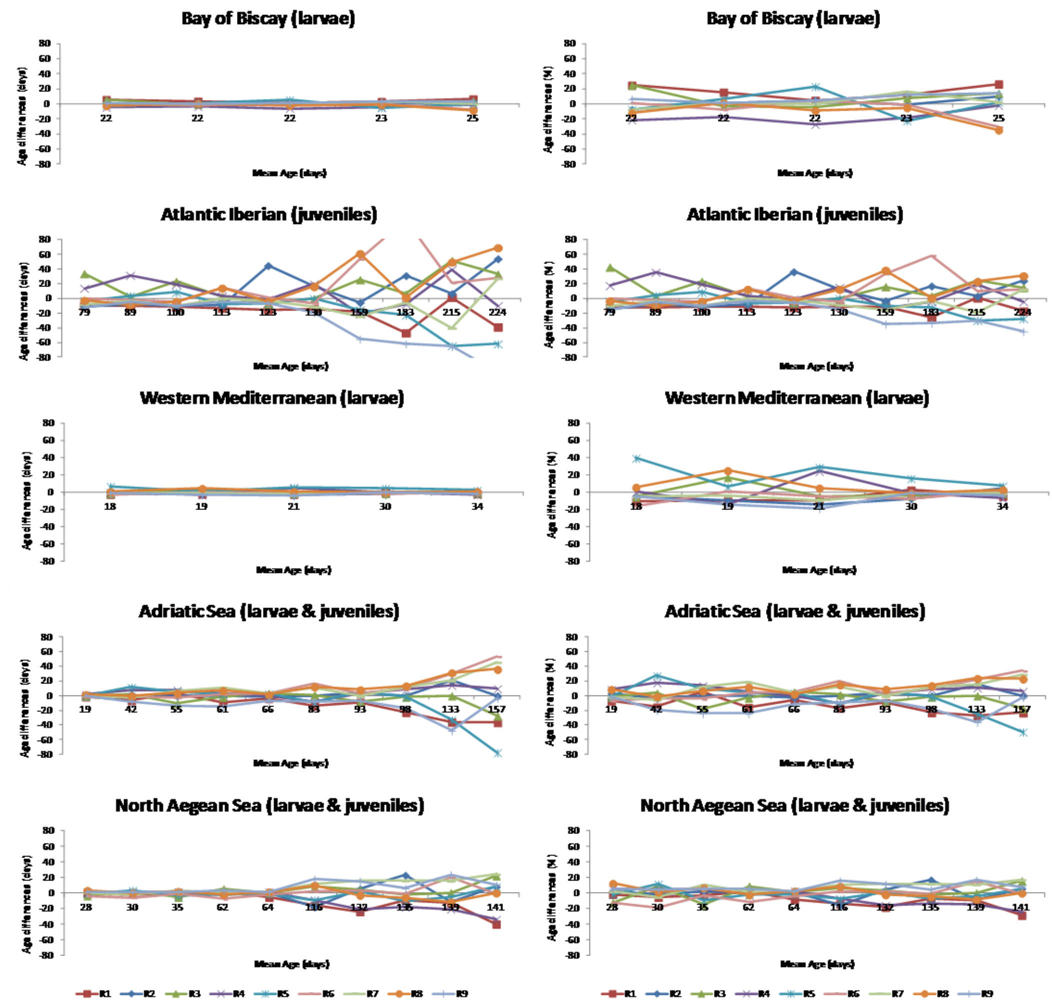


Figure 37 SARDINE: Age differences in days (left) and percentage (right) from the mean age by sampling areas and reader.

We compared the increment width reported by sardine readers R2, R3, R4, R5, R7 and R9 (the other readers not recorded the increments width), in order to know the readings interpretation from each reader in larvae and juveniles. For this, we choose the image otolith readings with high level of precision in the age determination of the all sets (Figures 38 to 40). In the larvae samples of the Bay of Biscay (Code sample: St 533 – 11; SL= 18.69 mm; Ageing precision: 9%CV and 6.2%APE) and Mediterranean Sea (Code sample: OTO 385-TF1110; SL= 22.9 mm; Ageing precision: 5%CV and 3.8%APE), all readers seem to apply the same reading criteria in the larvae, although it is noted great variability of the increments width among readers, especially in the first increments after hatching. The reader R2 also shows great variability of the remaining increments width (Figure 38)

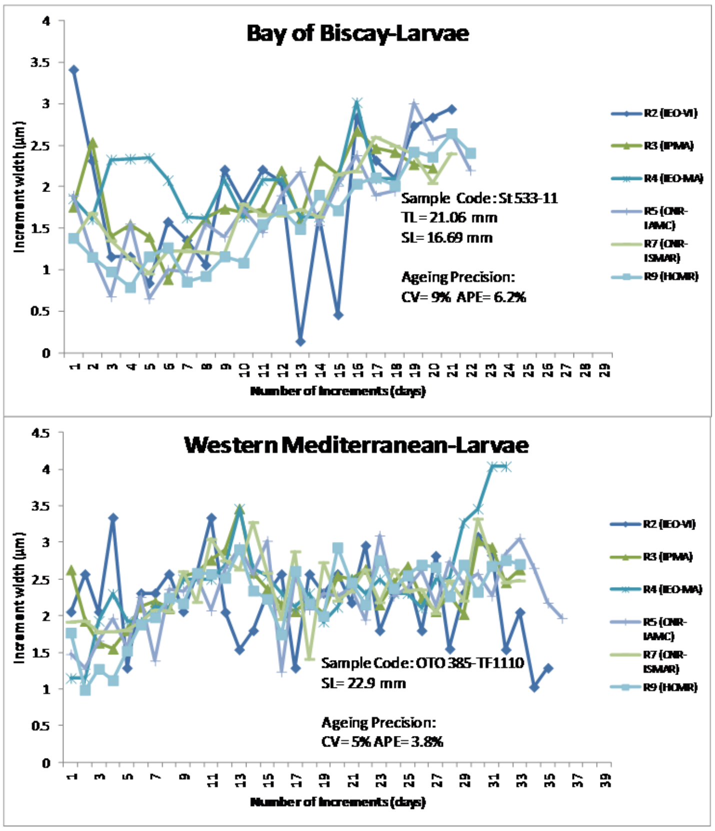


Figure 38. SARDINE. Comparison of the increments width results (by reader) from larvae of Bay of Biscay (top panel) and Western Mediterranean Sea (bottom panel). The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

In the juveniles sample of the Atlantic Iberian, high variability is observed in the increments width assigned by reader R2, R3 and R5 (Code sample: 27_05_04_07_200x SL= 71 mm; Ageing precision= 9%CV and 6.8%APE), and the other readers (R4, R7 and R9) appear to follow the same reading criteria (Figure 39).

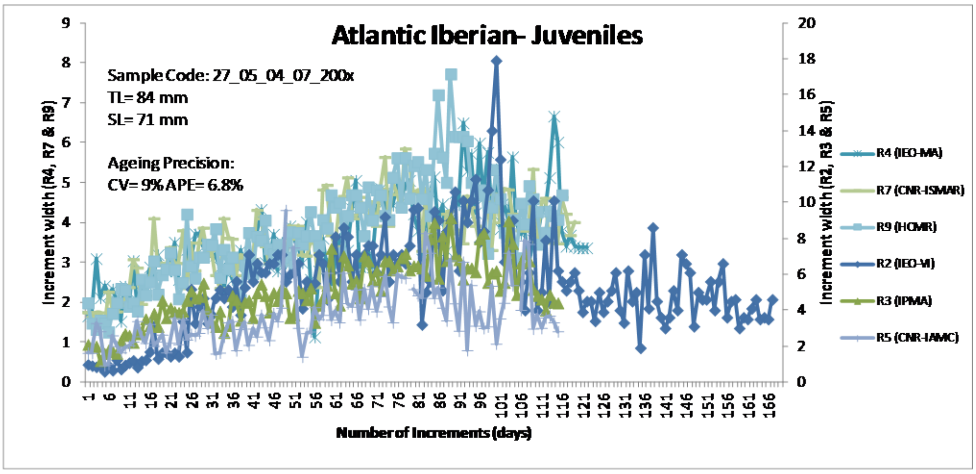


Figure 39. SARDINE. Comparison of the increments width results (by reader) from juveniles of the Atlantic Iberian (ICES Division IXa). The sample code, fish length (mm) and ageing precision (CVandAPE) are indicated.

In the samples of the Adriatic Sea, also variability is observed in the width of the first increments in the larvae (Code sample: Ms 174; SL= 14 mm; Ageing precision= 6% CV and 4.6%APE) among all readers, although seem to have the same reading criteria. The reader R7 seems to have had calibration problems in reading image. In juveniles (Code sample: MS82; SL=39 mm; Ageing precision= 7%CV and 5.8%APE), high variability is observed, though without any evident pattern of growth among readers (Figure 40). Some readers have noted that there were problems with the calibration of some images of this set, so they were not sure about radius and increment measurements from nucleus.

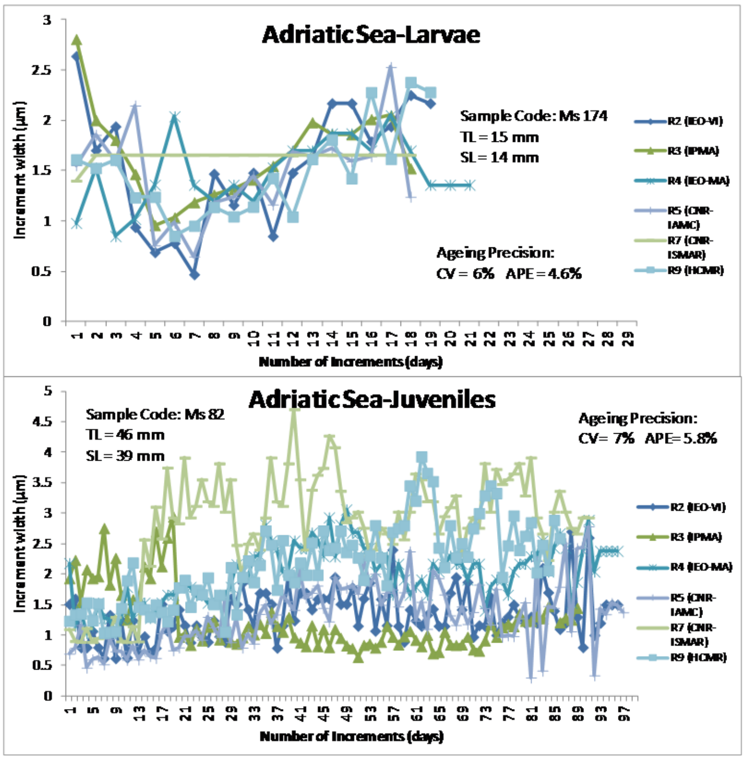


Figure 40. SARDINE Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel) of the Adriatic Sea. The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

In the samples of the North Aegean Sea, high similarity is observed in the width increments in the larvae (Code sample: Sp_L_2-3; SL= 34.4 mm; Ageing precision= 4% CV and 3%APE) among all readers. Generally readers adopted similar ageing criteria in the sample of juveniles (Code sample: Sp_J_10_9; SL= 59 mm; Ageing precision= 10% CV and 7.5%APE), although some variability is observed in the increments width of readers R2 and R5 (Figure 41)

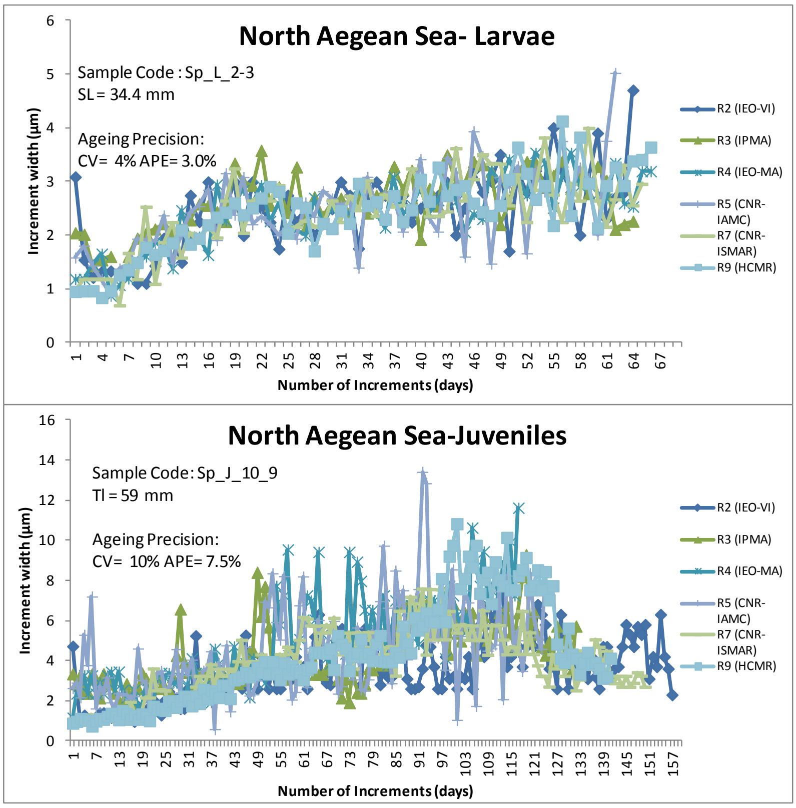


Figure 41 SARDINE Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel). The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

8.2.3 Aquaculture juveniles of Sardine from Atlantic Iberian set

Table 8.9 details length and month of landing of culture juveniles set (Atlantic Iberian) of otoliths images selected for the sardine exchange program with the ageing produced by each reader. The mean age is here replaced by actual age, since this fish is of known age (from aquaculture). The last four columns in the top table show actual age, standard deviation (SD) and precision of reading as the CV in relation to the actual age and the APE_{BF}; and in the bottom table shows also mean age, standard deviation (SD) and precision of reading as the CV in relation to the mean age and the APE_{BF}.

Table 8.9 SARDINE Otolith SET (WKMIAS 2013_Otolith Exchange): Aquaculture juveniles from Atlantic Iberian

Actual Age

Sample		Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Actual		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	70	27.8	40%	15.3%
2010	sc_180810_6_1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	85	11.1	13%	8.7%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	101	4.1	4%	11.6%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	116	8.3	7%	6.9%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	160	94.2	59%	18.5%
					Total read	5	5	5	5	5	4	5	5	5			24.6%	12.2%
					Total NOT read	0	0	0	0	0	1	0	0	0				

Mean Age

Sample		Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APE_{BF}
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	79	16.0	20%	15.0%
2010	sc_180810_6_1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	89	12.7	14%	9.4%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	100	13.5	14%	11.3%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	113	9.8	9%	6.8%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	130	14.5	11%	8.9%
					Total read	5	5	5	5	5	4	5	5	5			13.6%	10.3%
					Total NOT read	0	0	0	0	0	1	0	0	0				

In general, the exchange shows greater differences between readers when we relate it to the actual age (CV = 24.6%) than the mean age (CV = 13.6). The mean age is very similar to the actual age in three otoliths image, and therefore the difference between readers is very low with an ageing precision very high (CV between 4 and 13%). (Figure 42).

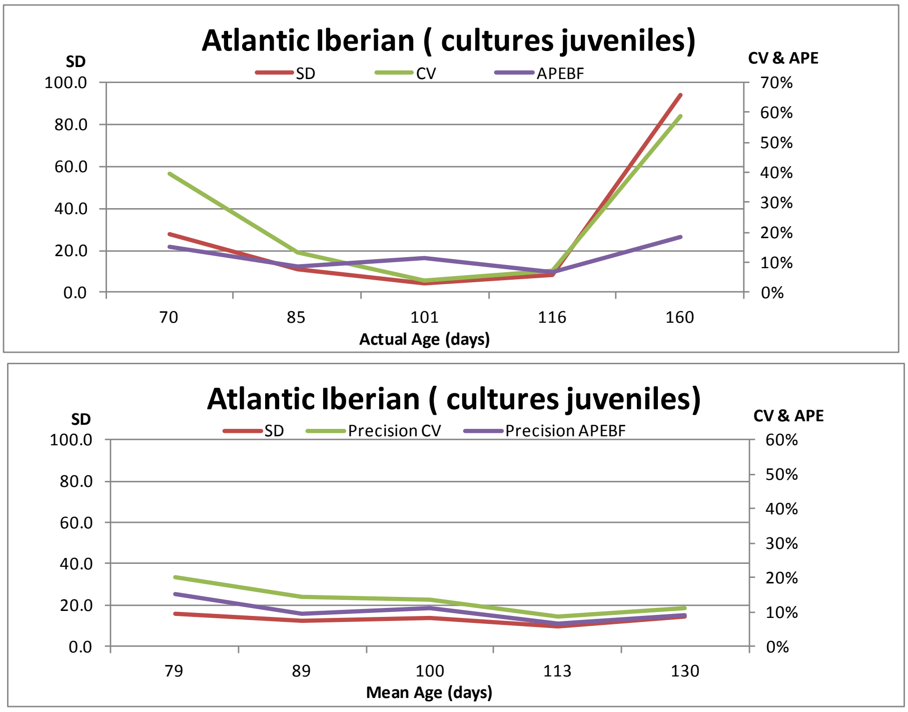


Figure 42. Aquaculture juveniles of SARDINE: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against ACTUAL age (top panel) and MEAN age (bottom panel).

In general, readers R1 and R9 underestimate the ages respect to actual age (difference of 13% and 11% from the actual age, respectively), and readers R3 and R4 overestimate them (14% and 17% respectively. (Table 8.10 and Figure 43). All readers underestimate the older fish (160 days), with differences between 8 and 49 days (between 6 and 31%) with the actual age.

Table 8.10. Aquaculture juveniles of SARDINE: Age differences (number of days and percentages) from the actual age.

Atlantic Iberian (culture juveniles)

Actual	IFREMER		IEO-VI		IPMA		IEO-MA		CNR-IAMC		CNR-ISMAR		CNR-ISMAR		CNR-ISMAR		HCMR	
age	R1		R2		R3		R4		R5		R6		R7		R8		R9	
70	-1	-1%	-2	-3%	43	61%	23	33%	6	9%	-	-	1	1%	7	9%	-3	-4%
85	-7	-8%	-4	-5%	6	7%	35	41%	7	8%	1	1%	0	0%	-6	-6%	-1	-1%
101	-13	-13%	-12	-12%	22	22%	18	18%	7	7%	-6	-6%	-11	-11%	-6	-5%	-11	-11%
116	-16	-14%	-12	-10%	0	0%	1	1%	-10	-9%	12	10%	-3	-3%	12	10%	-7	-6%
160	-44	-28%	-13	-8%	-30	-19%	-10	-6%	-30	-19%	-36	-23%	-41	-26%	-14	-8%	-49	-31%
Mean days	**-16**	**-13%**	**-9**	**-8%**	**8**	**14%**	**13**	**17%**	**-4**	**-1%**	**-7**	**-4%**	**-11**	**-8%**	**-1**	**0%**	**-14**	**-11%**

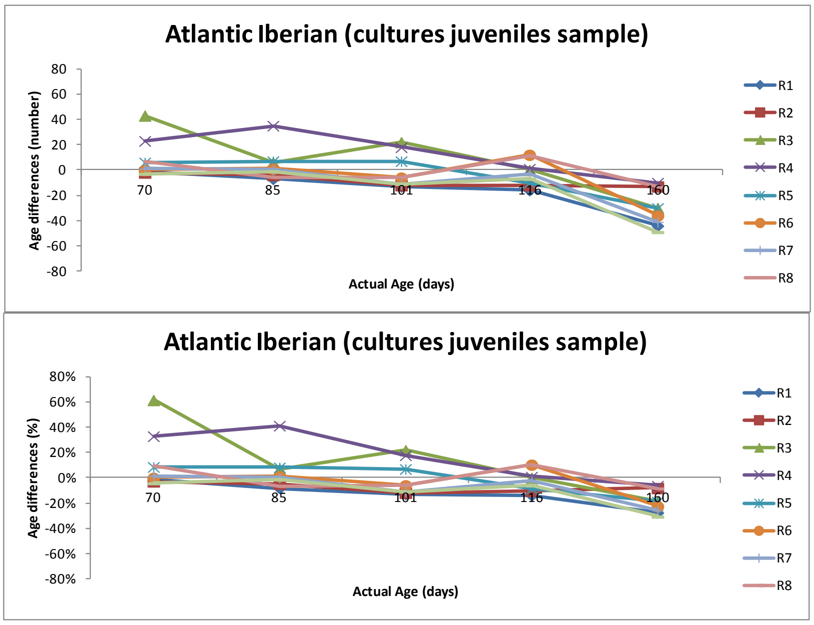


Figure 43. Aquaculture juveniles of SARDINE: Age differences in days (left) and percentage (right) from the mean age by sampling areas and reader.

Readers generally adopted similar ageing criteria in the culture juveniles sample (Code sample: sc_060910_2_1; SL= 67 mm; Actual Age= 101 days; Ageing precision= 4% CV), although some variability is observed in the increments width of reader R5 (Figure 44)

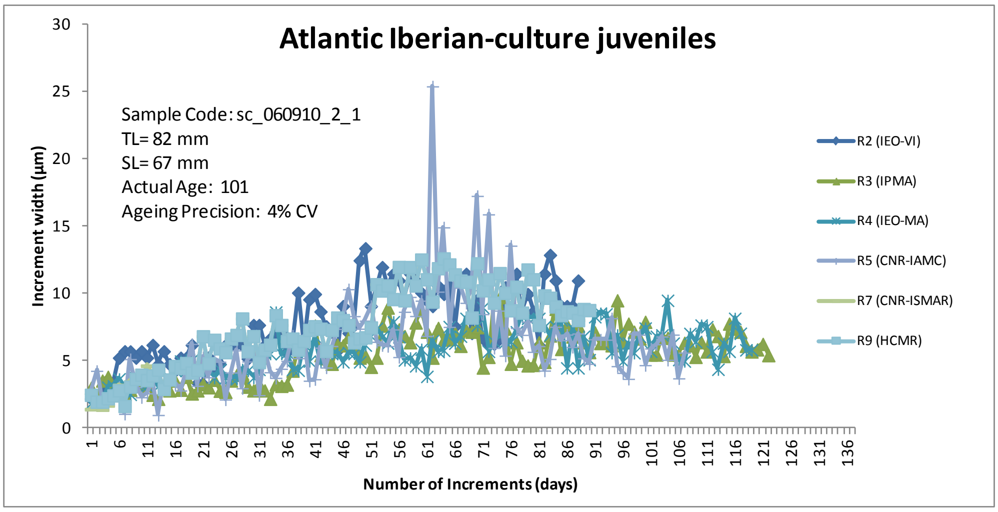


Figure 44. Aquaculture juveniles of SARDINE. Comparison of the increments width results (by reader). The sample code, fish length (mm) and ageing precision (CV and APE) are indicated.

8.2.4 Evaluation of the 2013 Anchovy and Sardine Otolith Image Exchange

This is the first Otolith Image Exchange that takes place in the ICES to compare the readings of daily micro increments among laboratories from different member states. The Exchange exercise showed differences among readers and areas for both species (Table 8.11).

Table 8.11 2013 Anchovy and Sardine Otolith Image Exchange: Summary of the precision (CV and APE) by areas and total.

Live Stage	Area	CV %		APE %	
		Anchovy	Sardine	Anchovy	Sardine
Larvae & Juveniles	Bay of Biscay	15.3	14.5	10.4	10.7
Juveniles	Atlantic Iberian	-	18.0	-	13.5
Juveniles	Aquaculture Atlantic	-	24.6	-	12.2
Larvae	Western Mediterranean	18.6	11.7	14.1	8.6
Juveniles	Strait of Sicily	34.9	-	23.0	-
Larvae & Juvenilles	Adriatic Sea	24.0	14.3	18.9	11.6
Larvae & Juvenilles	North Aegean Sea	9.0	9.4	7.8	7.4
	Total	**18.9**	**13.7**	**13.3**	**10.6**

Differences in the age determination were generally lower for sardine. However, in sardine, a greater variability was observed in the allocation and width of increments, suggesting that not all readers followed the same ageing criteria for this species, especially readers R2, R3 and R5 (and also probably R1). This could be because sardine readers have a low level of daily age expertise.

The comparison with the actual age of sardine showed that sardine readers are generally in good agreement, with a greater deviation of readers R3 and R4. Nevertheless, all readers underestimated the older fish (160 days).

In general, anchovy reader R5 tended to overestimate daily ages considerably in the anchovy images, showing clear differences in the age interpretation criteria, much more in the older ages (juveniles). Conversely, R9 tended to underestimate daily ages in the anchovy larvae. However, bearing in mind the inherent difficulties to interpret the daily micro increments in anchovy, generally most readers apply the same reading criteria in all areas.

Good quality images generally provided high ageing precision for both species, for example those from the North Aegean Sea. Therefore, it should be stressed the importance of obtaining clear images to properly interpret daily micro increments in this species.

The reasons that might explain the agreement and discrepancies in the anchovy and sardine exchange can be summarized as follows: a) Unclear images, in which was difficult to interpret well the pattern of daily growth due to under-or over-polishing, poor image acquisition or calibration problems; b) Difficulties in interpretation of subdaily increments, double structures or band zones (see Cermeño *et al*., 2006; Cermeño 2008); c) Different interpretation criteria.

8.3 Results of comparative daily age readings at the Workshop

8.3.1 Anchovy Results (see also annex 6)

Table 8.12 details length and month of landing of the otolith image selected for the anchovy (juvenile from Adriatic Sea Set) with the ageing produced by each reader during the workshop. The last four columns give mean daily age, standard deviation (SD) and precision of reading as the CV in relation to the average age and the APEBF.

This exercise showed that the precision was very high (CV = 3% and APE= 2.4%) with respect to the number of micro increments assigned. We found that all readers have a significant agreement in the readings (the mean counts differ <5% from the mean age; Table 8.13). All readers were experts in the age reading of juvenile anchovies and all of them were involved also in the exchange. These results indicate a noticeable improvement in age precision compared with the exchange results (that gave a CV of 14% and an APE of 8.2% in this same sample)

Table 8.12 ANCHOVY Otolith from Adriatic Sea (WKMIAS 2013_ Otolith Intercalibration at the Workshop).

Sample year	Sample no	TL (mm)	SL (mm)	Landing month	AZTI R2	IEO-ST R3	CNR-IAMC R5	CNR-ISMAR R6	HCMR R9	Mean age	SD	Precision CV	Precision APE_{BF}
1996	E666	46	39.0	12	75	70	75	72	74	73	2.2	3%	2.4%

Table 8.13 ANCHOVY Otolith from Adriatic Sea: Age differences (number of days and percentages) from the mean age by reader.

Mean age	AZTI R2		IEO-ST R3		CNR-IAMC R5		CNR_ISMAR R6		HCMR R9	
73	2	2%	-3	-4%	2	2%	-1	-2%	1	1%

However, to analyse in detail the position of the rings assigned by each reader, there were differences in zones of the otolith that exhibited greater complexity. That is, areas where there are double rings or areas where the rings are not well marked. In these zones are differentiated the two reading criteria (GBR and IMR) used by the readers, as detailed in Figure 45. The reader R5 and R6 apply the IMR reading criteria, while the rest of the readers follow the GBR criteria.

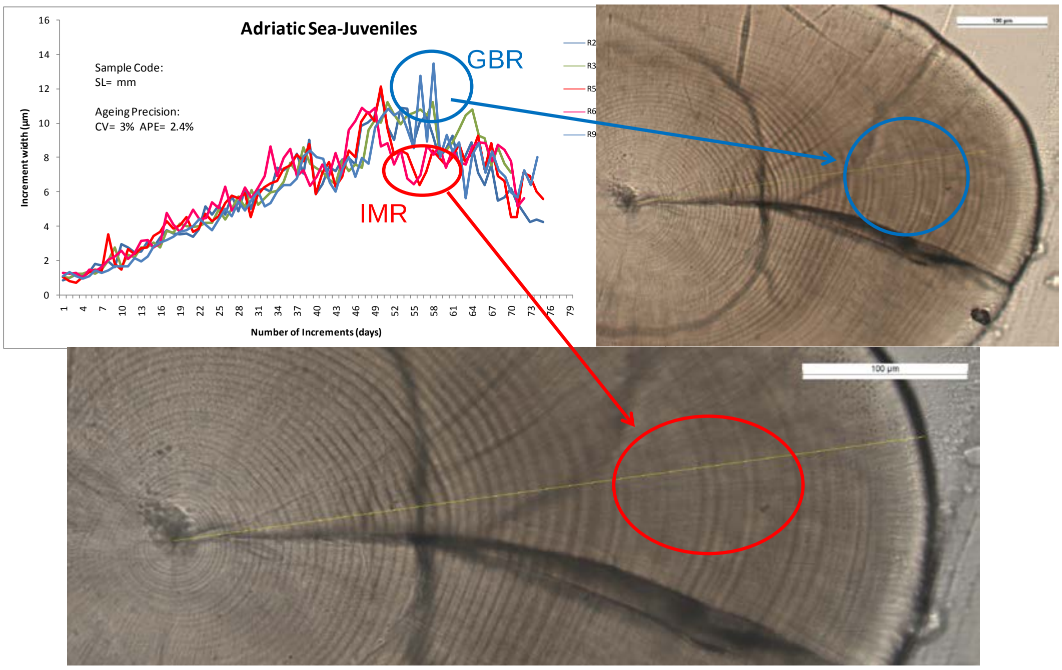


Figure 45. ANCHOVY Otolith from Adriatic Sea. Comparison of the increments width results (by reader).

8.3.2 Sardine results (see also Annex 7)

Table 8.13 details length and month of landing of the otolith image selected for the sardine (juvenile from Aquaculture of Atlantic Iberian) with the ageing produced by each reader during the workshop. The mean age is here replaced by actual age, since this fish is of known age (from aquaculture). The last four columns in the top table give actual daily age, standard deviation (SD) and accuracy of reading as the CV in relation to the actual age and the APE_{BF}; and in the bottom table shows also mean age, standard deviation (SD) and precision of reading as the CV in relation to the mean age and the APE_{BF}.

This exercise showed that the accuracy was high (CV = 10% and APE= 6.7%) with respect to the number of micro increments assigned. These results indicate a noticeable improvement in age accuracy compared with the exchange results (that gave a CV of 40% and an APE of 15.3% in this same sample). The specimen had a known age of 70 days. The mean age (67) is very similar to the actual age in this otolith image (< 1% deviation from the actual age).

Table 8.13 SARDINE Otolith from Aquaculture of Atlantic Iberian (WKMIAS 2013_Otolith Intercalibration at the Workshop)

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IPMA R3	CNR-IAMC R5	CNR-ISMAR R7	Actual age	SD	Precision CV	Precision APE_{BF}
2010	sc_040810_6_1	1	67	54.2	8	63	65	72	70	7.1	10%	6.7%

Sample year	Sample no	Fish no	TL (mm)	SL (mm)	Landing month	IPMA R3	CNR-IAMC R5	CNR-ISMAR R7	Mean age	SD	Precision CV	Precision APE_{BF}
2010	sc_040810_6_1	1	67	54.2	8	63	65	72	67	4.7	7%	5.3%

We found that all readers have a significant agreement in the readings (the counts differ <10% from the actual age; Table 8.14). In this case, readers of sardines were only three at the workshop (only those usual readers of juvenile sardine), an expert reader (R7) and two readers with low experience (R3 and R5). The three readers were also involved in the exchange. The reader R3 considerably improves respect to the exchange (that assigned 113 days in the exchange and 63 days at the workshop). The reader R5 underestimates at workshop (65 days) and overestimate in the exchange (76 days) and reader R7 shows similar results with respect to the exchange (71 at the workshop and 72 days in the exchange).

Table 8.14 SARDINE Otolith from Aquaculture of Atlantic Iberian: Age differences (number of days and percentages) from the actual age by reader.

Actual age	IPMA R3		CNR-IAMC R5		CNR-ISMAR R7	
70	-7	-10%	-5	-7%	2	3%

Readers generally adopted similar ageing criteria in the aquaculture juveniles sample, although some variability is observed in the increments width among readers, over all the reader R5 (Figure 45).

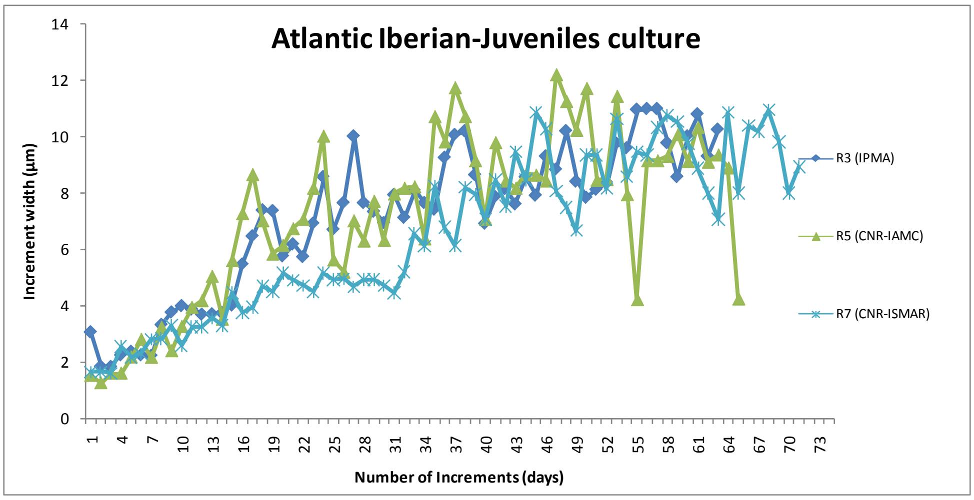


Figure 45 SARDINE Otolith from Aquaculture of Atlantic Iberian. Comparison of the increments width results (by reader).

8.3.3 Evaluation of the Anchovy and Sardine Otolith Workshop

Comparing the results of the first and the second daily age reading it is clear a considerable improvement for both species and for most readers, although it should be taken with caution as it was only possible compare one otolith image of each species. Only two readers involved in the exchange did not participate in the workshop. There was a large improvement for sardine reader R3 and for anchovy reader R5.

In the case of anchovy, although the precision was improved, there was much discussion in the allocation of micro increments in those zones of the otolith with the presence of double bands. This was done by reading juvenile otolith image from the Adriatic Sea, as readings of the exchange had presented a high level of discrepancy in this area and besides during the workshop not reached a unanimous agreement on the reading criteria in the samples from the Adriatic Sea (See section 7.3). Therefore readers applied different reading criteria in these parts of the otolith more complex. Thus, it is recommended validation studies of the daily periodicity of the growth increments in the otoliths of juveniles in those geographic areas that have not been performed yet.

Sardine results indicate a significant improvement, both in accuracy and in the allocation of micro increments. This was done by reading otolith image of known age (obtained from aquaculture), and the accuracy of the age readings can be seen and thus could validate the technique of reading. Unfortunately, very few samples of known age were available, so it is recommended that in future can be provided a collection of otoliths of known age to validate the ageing technique of this species.

9 Reference collection of otolith images

The otolith select images by species and from different areas were presented during the exchange and workshop. For reference collection, several criteria were taken:

Engraulis encrasicolus

- We selected aged otolith images in which the precision of the age determination among readers was high (CV ≤10%) in the exchange collection. This is the case of the reference collection of the Bay of Biscay (2 larvae and 2 juveniles), Western Mediterranean (2 larvae) and North Aegean Sea (2 larvae and 2 juveniles).
- In cases of Strait of Sicily and the Adriatic Sea new images were selected, in which age was determined by the most experienced reader within each area. This was because in these areas there were no otolith images with high precision levels of the age determination among readers in the exchange. This is the case of reference collection of the Strait of Sicily (2 juveniles) and Adriatic Sea anchovy (2 larvae and 2 juveniles).

Sardina pilchardus

- We selected aged otolith images in which the precision of the age determination among readers was high (CV ≤10%) in the exchange collection. This is the case of the reference collection of the Bay of Biscay (2 larvae), Western Mediterranean (2 larvae), North Aegean Sea (2 larvae and 2 juveniles), Atlantic Iberian (2 wild juveniles and 2 aquaculture juveniles). Juveniles from aquaculture had a known age.
- In cases of Adriatic Sea new images were selected, in which age was determined by the most experienced reader within each area. This was because in these areas there were no otolith images with high precision levels of the age determination among readers in the exchange. This is the case of reference collection of the Adriatic Sea (2 larvae and 2 juveniles).

The reference collection of otolith images produced during the workshop is presented in annex 5. In each image, the characteristics of the sample and the mean age obtained on exchange are detailed.

10 Contributions to the Workshop (abstracts)

WD 01: Aldanondo, N., Cotano, U., Alvarez, P. and Uriarte, A., 2013. Validation of annual increment deposition in the otoliths of young-of-the-year European anchovy

In order to validate annual increment deposition in the otolith of European anchovy, early anchovy juveniles were captured in October 2012 in the southern Bay of Biscay. These individuals were maintained in captivity in a spherical culture cage (radius 16 m, 4 mm mesh size) until April 2013. Fifty juveniles were sampled at around 27-day intervals and measured to the nearest mm for standard length (SL). Otoliths were removed from each sample and processed following the methodology described in Aldanondo *et al.*, (2010).

Briefly, the central part of juvenile otoliths was read at x1000 magnification in immersion oil and the outer part was analysed at x100. For the translucent band analysis, x630 magnification was used. All increments were counted starting at hatch increment (Aldanondo *et al.*, 2008) and the distance between increments was measured along the same axis from the core to the edge of the otolith in the post-rostrum side. For daily increment interpretation, the group band reading (GBR) method suggested by Cermeño *et al.* (2008) was followed. As daily increment deposition has been validated for European anchovy larvae (Aldanondo *et al.*, 2008) and juveniles (Cermeño *et al.*, 2003), the increment number was considered a proxy for age in days.

From October 2012 to January 2013, anchovy juvenile lengths increased slightly or remained stable at around 98 ±6.3 mm (mean ±S.D). After this period, SL significantly increased up to the mean value of 120 mm in April 2013. Likewise, the age (in days) of anchovy juveniles was estimated based on otolith microstructure analysis. The estimated age varied from 100 (for individuals sampled in October 2013) to 293 days (for anchovies sampled in April 2013). The general otolith daily growth pattern showed that increment widths increased rapidly and were broadest between 62 and 68 days, with mean ±S.D of 20.1 ±6.2 μm. Thereafter, the widths decreased steadily to 1.5 μm. This study also revealed that, in the case of European anchovy in the Bay of Biscay, the first translucent band formation started in October-November and it was completed in April.

WD 02: José Iglesias and Lidia Fuentes 2013 (presented within the WKMIAS by Isabel Riveiro). Culture viability of *Sardina pilchardus*: preliminary results of growth in captivity.

Larvae of *Sardina pilchardus* were obtained in captivity from fertilized eggs captured in the wild, and grown in a 10 000 L tank. *Isochrysis galbana*, *Artemia franciscana* nauplii and live zooplankton were used as prey during the first two weeks; afterwards, animals were fed on *artemia metanauplius* enriched with *Isochrysis galbana*. A dry feed (Gemma 0.4 and 0.8) from SKRETTING S.A. (Burgos, España) was supplied from the third to 18th month. The total length reached by sardines at one year of life was 162.02 ±9.49 mm, corresponding to a wet weight of 36.12 ±10.82 g. Total length at 18 months was 182.37 mm. A long experiment (18 month) of sardine culture is described for the first time, and growth data reported can contribute to determine its potential interest as a candidate for marine aquaculture

WD 03: C. Hernández, B. Villamor, J. Barrado, C. Dueñas, S. Fernández Age determination in European anchovy (*Engraulis encrasicolus* L.) otoliths in the Bay of Biscay (NE Atlantic).

This study arises from the two recommendations given in the Workshop on Age reading of European anchovy, WKARA 2009, due to the difficulties encountered by various experts readers in annual age to determinate between first annual ring and the check formed before its first winter ring. Two methods were used, in the first, age was determined by identifying and measuring growth rings formed on sagitta otoliths (Morales-Nin, 1988). In order to support the identification of the first annual ring, the otolith radius of the first hyaline ring was measured and used as a gauge for exclude the first check in ageing older individuals. In the second, a method for age corroboration was used by means of the otolith microstructure and fish ages were determined by daily increment counts (Campana *et al*., 1985; Campana, 2001). Total number of daily increments in otoliths was counted to test whether identified macroscopically hyaline area is, in fact, a check or the first annulus (Waldron *et al*., 1998, Waldron *et al*., 2001). We compare the results of age determination using whole otoliths with those determined through counts of daily increments in an attempt to corroborate the above method.

The results obtained through the analysis of otolith microstructure indicated that the hyaline zone macroscopically identified as a check is not an annual growth area because they had less than 365 daily increments in otoliths before deposit of this ring. In cases where the distance from the core to the first visible ring was <850 ±100 μm, this ring was assigned by the reader as a presumed check.

This study has confirmed that the estimated ages read using whole otoliths with those determined through counts of daily increments are generally correct up to the age of one year, supporting that the reader can distinguish correctly between rings and false rings and fish can be assign to the correct year class.

Based on the corroboration of age estimates presented here two recommendations to annual readers for ageing routines has been established:

1) It is recommended to anchovy annual readers to measure the distances between the core and the inner edge of the hyaline rings, it is usually well marked in the otoliths and can be measured accurately.

2) From the validation of daily ring formation in anchovy larvae and juveniles (Cermeño *et al*., 2003; Aldanondo *et al*., 2008), and corroboration of the position of the first hyaline ring (check) formed before their first winter annual ring, through the counts of micro-increments, is suggested to measure 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.

WD 04: C.Hernández[1], I.Riveiro[2], E.López[2] Problems found with Exchange otolith Images

A presentation about the problems found by sardine and anchovy readers with exchange otolith images was made. In some images the core was not exposed, therefore the length of the total radius in the post-rostrum axis cannot be measured, also the increment widths in this area can vary, so interpolation would be inaccurate. Several calibration problems were also found, images with the scale inserted incorrect, mosaic images without scale inserted, objective micrometre images without units. Other mosaic images were not uploading in the SharePoint, the mosaic (two or three images for one sample) was to be made by each reader. In other images were not possible to identify the growth pattern (GBR) and some otoliths were rejected because more than 10% of the increment count was only possible by interpolation.

WD 05:C. Hernández, B. Villamor, J. Barrado Laboratory protocol for processing sagittae from juvenile anchovy

Removal , Cleaning and Selection

Record data from the fish sample (i.e. length and weight)

Remove otoliths sagittae from fish

Rinse with distilled water. Place otolith in an tray

Select the otolith. Discard broken otoliths and those who show no calcification areas

Sectioning

To obtain sections is important that all include the core to prevent the loss of the sequence of increases and thus avoid underestimation of age. The most appropriate section plane in the sagittal otolith of anchovy is the sagittal plane (Morales-Nin, 1992).

Sanding

Heat a clean slide on a hot plate (SBS-Cpc 135 D) to a temperature of 135 ºC .

Place a small piece of hard Crystal Bond (Mounting wax 40-8150) on the slide and allow it to melt.

Place the otolith on the glue. The face of the otolith that sticks first is the proximal face, with the sulcus down.

Otoliths were then saund on both sides with a Buehler Metaserv rotating wheel covered with a grinding paper (grit 3 µm for smaller otoliths and 5 µm for bigger ones) at 200-500 rpm until nucleus and primary growth increments were visible. A slow and cautious approach should be adopted in order to prevent the over-grinding of the otolith. Regular checks of sanding plane should be made throughout preparation to prevent such an occurrence.

Storage of sections

Store polished sections (attached to glass slides) in slide boxes. Label slide boxes with appropriate sample information.

Images analysis

It is useful to obtain digital images of the whole otolith preparation at this point as an aid to orientation for analysis. This acquisition system and image processing consists of three units:

An image acquisition unit (camera): Sony DFW-SX910 microscope connected to a Nikon Eclipse 50i with 5 targets from 4x to 100x magnification.

A central processing unit, storage and visualization: a team with a large random access memory (minimum 2 GB) for digital analysis with 2 screens (Screen 1: display calcified piece, screen 2: Biological parameters or analysis image). A software unit: TNPC 4.1 software (VisiLog 6.4) developed by Ifremer with the company Noesis. It is used to acquire and interpret images of objects calcified, particularly otoliths for age determination.

WD 06: J.M. Quintanilla and A. García. Estimating Daily Age of anchovy larvae using discriminant function analysis

Age estimation by otoliths is paramount to fisheries science. Otoliths are the most reliable bony part in fish to estimate age, and particularly in larvae by the analysis of their microstructure (Campana y Neilson, 1985; Secor *et al.*, 1995, Campana, 1999). This implies a meticulous process of extraction and mounting of otoliths. The complexity of the process consumes time and the interpretation of daily increments requires expert qualifications (Megalofonou 2006). As a result, age determination in larval fish implies a high cost/benefit analysis per otolith (Bedford, 1983; Cardinale and Arrhenius 2004, Francis and Campana 2004).

Since the seventies with the development of computer software developments, semi-automatic methods were designed to estimate the age from otoliths (Manson, 1974). The objective was twofold: on one hand, to reduce time-consuming tasks in the interpretation of otoliths and on the other part, reduce the subjectivity from age reader's interpretation. (Francis y Campana, 2004).

Discriminant function analysis allows classifying individuals of unknown origin into groups by using discriminant functions generated from a database of information of individuals of known origin (McGarigal *et al.*, 2000). The database comprised somatic and biometric data from larvae whose age was estimated by an expert reader. This constituted the "training sample" from which discriminant functions are obtained for assigning age to larvae of unknown age (test sample) (Francis and Campana, 2004), allowing the inclusion of variables for attaining discriminant functions.

This technique was applied on Mediterranean anchovy larvae with the purpose of:

determining which are the best age-correlated variables in anchovy larvae;

constructing a predictive model that allows to estimate age with a high percentage of correct assignments based on somatic and otolith biometry

And, thus the implementation of discriminant functions is aimed to eliminate subjectivity of age readers, which may be considered a major source error between different age reader's estimates; and furthermore, increasing the repeatability of age estimates. The implementation of the technique implies a lower cost/benefit analysis per otolith reading.

WD07: Villamor, B., G. Basilone and M. La Mesa. 2013. Anchovy and Sardine Micro increment daily growth exchange results.

Before the workshop on daily age reading (ICES WKMIAS 2013), it has been considered useful to plan an exchange programme of anchovy and sardines otolith images in order to ascertain the current level of precision among Institutes and the difficulties that the daily age reading of anchovy and sardine otoliths present. The exchanged otolith collection included 81 images (41 for anchovy and 40 for sardine) distributed in 10 sets from different anchovy and sardine distribution areas (Bay of Biscay, Atlantic Iberian Peninsula, Western Mediterranean, Strait of Sicily, North Adriatic Sea and North Aegean Sea). In the case of sardine, also a small otoliths collection (5) of known age (obtained from Aquaculture) was used. The exchange proved the existence of differences between readers and areas of both species, with a precision ranging from 9.0 to 34.9% CV for anchovy, and from 9.4 to 18.0% CV for sardine. Differences in the age determination were generally lower for sardine. However, in sardine, a greater variability was observed in the allocation and width of increments, suggesting that not all readers followed the same ageing criteria for this species. The comparison with the actual age of sardine showed that sardine readers are generally in good agreement; nevertheless all readers underestimated the older fish. Good quality images generally provided high ageing precision for both species, for example those from the North Aegean Sea (9.0% CV for anchovy and 9.4% for sardine). Therefore, it should be stressed the importance of obtaining clear images to properly interpret daily micro increments in this species. The reasons that might explain the agreement and discrepancies in the anchovy and sardine exchange can be summarized as follows: a) unclear images, in which was difficult to interpret well the pattern of daily growth due to under-or over-polishing, poor image acquisition or calibration problems; b) Difficulties in interpretation of sub daily increments, double structures or band zones.

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Finally many thanks to all the participants which made a very big effort in order to fulfil the terms of references of the workshop in the available time. Their contributions also allowed the making of this report.

Annex 1: List of participants

Workshop on Micro increment daily growth in European Anchovy and Sardine (WKMIAS)

Mazara del Vallo, Sicily
21–25 October 2013
Chairs: G. Basilone, Italy,
Begoña Villamor, Spain
Mario La Mesa, Italy

Participants List

	Name	Country	E-mail
1	Andreia Silva	PT	avsilva@ipma.pt
2	Begona Villamor	ES	begona.villamor@st.ieo.es
3	Carmen Hernandez	ES	carmen.hernandez@st.ieo.es
4	Eliza Domenella	IT	elisa.domenella@an.ismar.cnr.it
5	Elise Bellamy	FR	elise.bellamy@ifremer.fr
6	Eudoxia Schismenou	GR	schismenou@her.hcmr.gr
7	Fortunata Donato	IT	f.donato@ismar.cnr.it
8	Isabel Rivero	ES	isabel.riveiro@vi.ieo.es
9	Jose Maria Quintanilla	ES	jose.quintanilla@ma.ieo.es
10	Mario La Mesa	IT	m.lamesa@ismar.cnr.it
11	Monica Panfili	IT	m.panfili@ismar.cnr.it
12	Naroa Aldanondo	ES	naldanondo@azti.es
13	Salvatore Gancitano	IT	salvatore.gancitano@iamc.cnr.it
14	Salvatore Mangano	IT	salvatore.mangano@iamc.cnr.it
15	Tomaz Modic	SI	Tomaz.Modic@zzrs.si
16	Sergio Vitale	IT	sergio.vitale@iamc.cnr.it
17	Gualtiero Basilone	IT	gualtiero.basilone@iamc.cnr.it
18	Maurizio Pulizzi	IT	maurizio.pulizzi@iamc.cnr.it
19	Beatriz Beldarrain	ES	bbeldarrain@azti.es
20	Carmen Pineiro Alvarez	ES	carmen.pineiro@vi.ieo.es
21	Ignazio Fontana	IT	ignazio.fontana@iamc.cnr.it
22	Giovanni Giacalone	IT	giovanni.giacalone@iamc.cnr.it

Annex 2: WKMIAS final Agenda

ICES ACOM

Workshop on Micro Increment daily growth in european Anchovy and Sardine [WKMIAS]

Working group for Data Collection Regulation

Institut for Marine and Coastal Environment – National Council for Research, (IAMC-CNR),

Via L. Vaccara, 61Mazara del Vallo (TP),

21-25 October 2013

<u>Agenda</u>

Monday, 21 October

09:30	Opening of the meeting (Co-Chairs: Gualtiero Basilone, Italy, Mario La Mesa, Italy, and Begonia Villamor, Spain); invited speakers: Welcome, local arrangements, incl. computer and network arrangements, participants' introduction.
10:00	Introduction; Adoption of the Agenda; Brief overview of ToR's; Table of report contents and allocation of responsibilities
10:30	*Coffee break*
10:45	Review literature and consider recent research to define daily increment patterns in anchovy and sardine: each participant would contribute to define a list of references both on methodological approach adopted to daily increment counts and validation of annual rings
12.00	Presentation of the common protocol adopted for otolith preparation and results from the otolith exchange program: comparisons of precision against modal age and bias in age readings. Evaluate current levels of ageing agreement (precision) among institutes. Identify major difficulties in age determination producing observed disagreements
13:00	*Lunch break*
14:00	Compare and discuss differences/similarities among areas/stock in the otolith ring formation for anchovy and sardine reported from different laboratories
16:00	*Coffee break*
16:15	Produce a common otolith preparation and age reading protocol: review the adopted protocols from different laboratories to agree for a common intercalibration exercise to be done in the following meeting days (Tuesday or Wednesday) Review and discuss the produced documents on the anchovy and sardine otolith preparation/reading standardization

18:00	*End of session*

Tuesday, 22 October

9:00	Validation of anchovy and sardine annual ring patterns laid down in the first year of life according to the discussed criteria and to the intercalibration exercise results
10:30	*Coffee break*
10:45	Anchovy exercise of live readings to define and standardize the daily age reading criteria among areas.
13:00	*Lunch break*
14:30	Sardine exercise of live readings to define and standardize the daily age reading criteria among areas.
16:30	*Coffee break*
16:45	Continue exercise of live readings to define and standardize the daily age reading criteria among areas.
17:30	Results from exercise of live readings in the different laboratories
18:00	*End of session*

Wednesday, 23 October

9:00	Results from exercise of live readings in the different laboratories
10:30	*Coffee break*
10:45	Discussion on inter-calibration laboratory exercises concerning otolith daily ring counts (adding material for reference collection) planned to evaluate differences among readers/laboratories
13:00	*Lunch break*
14:30	Inter-calibration laboratory exercises anchovy and sardine from acquaculture
16:30	*Coffee break*
17:00	Results of the intercalibration exercize anchovy
18:00	*End of session*

Thursday, 24 October

9:00	Results of the intercalibration exercize sardine and discussion on the protocols agreement.
10:30	*Coffee break*
10:45	Discussion on growth-rate patterns of juvenile anchovy and sardine from different areas/environments observed during the exercise
13:30	*Lunch break*
14:30	Create a reference collection of otoliths and start the development of a database of otolith images

17:00	End of Session

Friday, 25 October

9:00	Recommendations based on the workshop results
10:30	*Coffee break*
10:45	Final Report structure and assignment of responsibilities among participants
11:45	Planning future activities for enhancing quality in anchovy and sardine age determination
13:00	*End of meeting*

Annex 3: Recommendations

3) It is recommended to annual ring readers to measure the distances between the nucleus and the inner edge of hyaline rings, this is usually well marked in otoliths and can be measured accurately.

4) Based on the validation of daily ring formation in anchovy larvae and juveniles (Cermeno *et al.*, 2003; Aldanondo *et al.*, 2008), and the corroboration of the position of the false annual ring (check) formed before its first winter ring, through the micro increment counts, it is suggested to measure the first hyaline ring and all the translucent rings laid down before a distance from the primordium of less than 850 ±100 microns should be considered as checks. As reference point, in Bay of Biscay anchovy the first annual ring would be at 1155.7 ±69.6 microns.

5) Based on the validation of daily ring formation in sardine larvae and juveniles (Alemany and Alvarez, 1994), and the corroboration of the position of the false annual ring (check) formed before its first winter ring, through the micro increment counts, it is suggested to measure the first ring and all the translucent rings laid down before a distance from the primordium of less than 1000 microns should be considered as checks.

6) Alternative methods for the estimation of age by discriminant functions based on somatic variables and otolith biometry in order to reduce subjective factor and cost/benefit ratio related to direct readings should be considered.

7) Is strongly recommended to use a "collection of known age" otoliths in order to check and calibrate the age estimations of readers.

8) Evaluate by further studies the seasonal variability of the growth micro-increments pattern in both species (anchovy and sardine) focused mainly on the recruitment period.

9) Validation should be carried out to progress in the ageing criteria by conducting more studies on the life history events of the fish. Such studies have to be carried out by means of direct mesocosm experiments.

10) A workshop should be convened in 2017 since some ageing difficulties still remain, mainly for some areas.

Annex 4: WKMIAS terms of reference for the next meeting

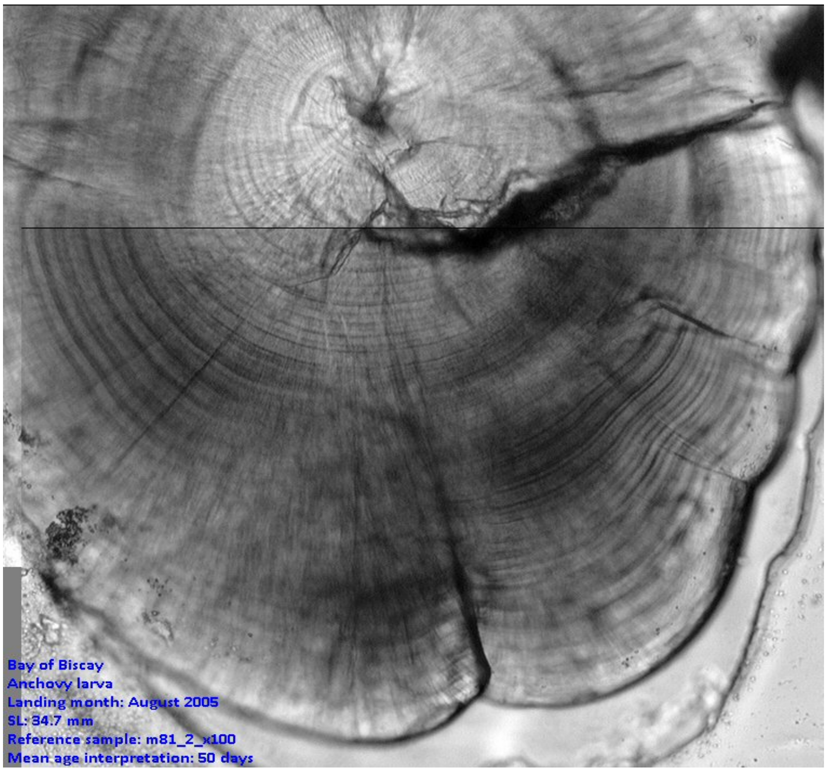
The **Workshop on Micro increment daily growth in European Anchovy and Sardine [WKMIAS]** - October 2017.

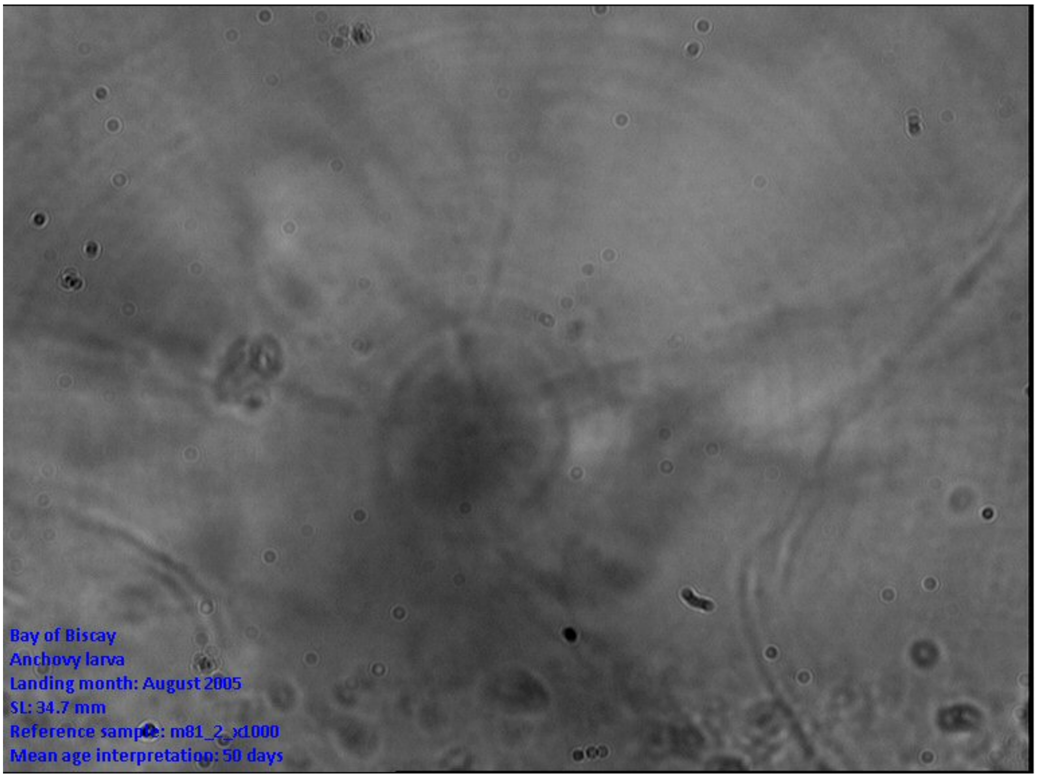
a) Review validation of daily ring formation;
b) Define and standardize the daily age reading criteria among areas;
c) Validate the first *annulus* in young of the year anchovy and sardine in different areas;
d) Estimate precision and accuracy of age estimates by micro-increment counts;
e) Improve the reference collection of otoliths created in the WKMIAS and start new collection of age known otoliths images;
f) Evaluate the reliability of new age assignment techniques (i.e. estimation of age by discriminant functions analysis).

Annex 5: Reference collection of otolith images

Engraulis encrasicolus larva

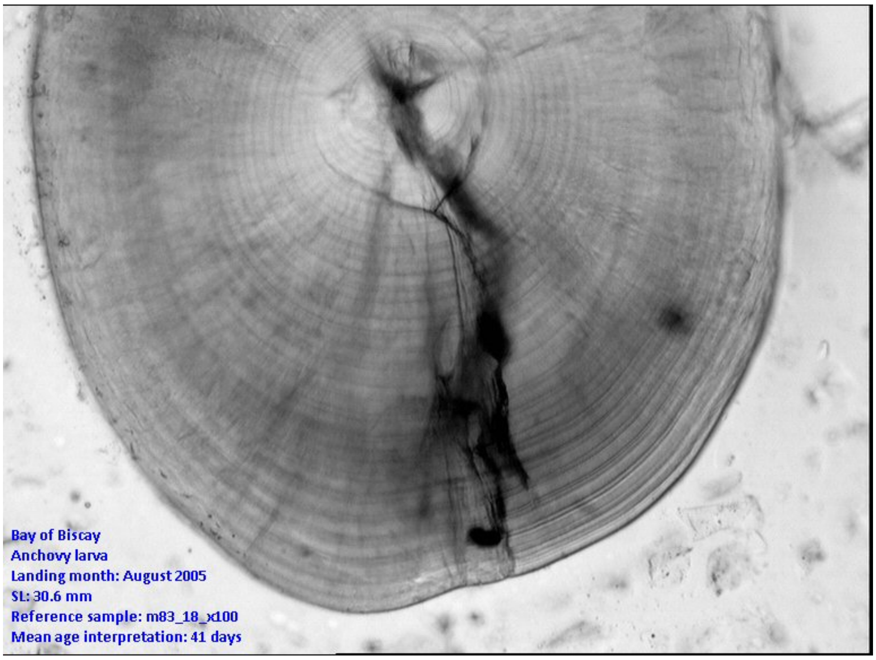
Bay of Biscay

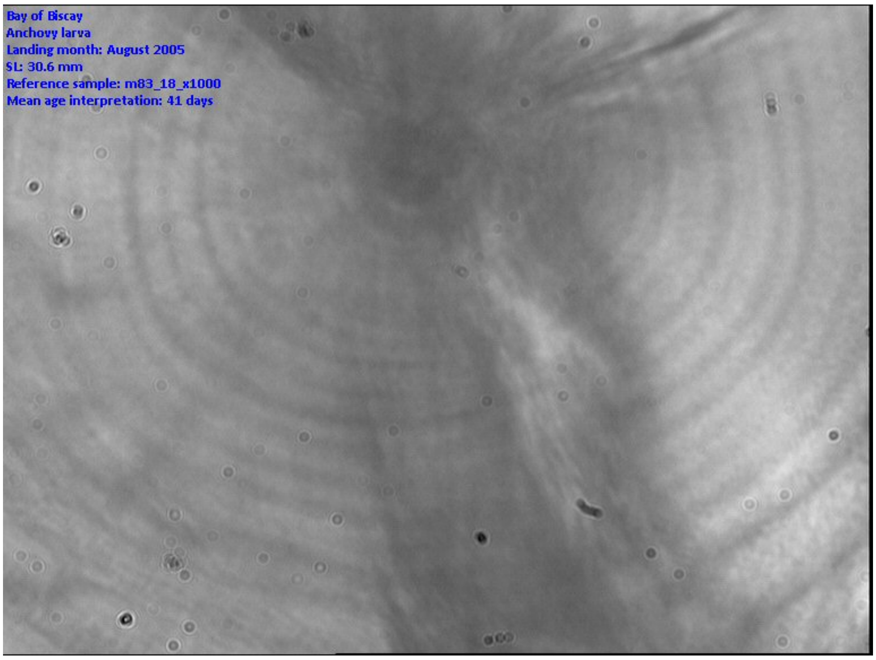




Engraulis encrasicolus larva

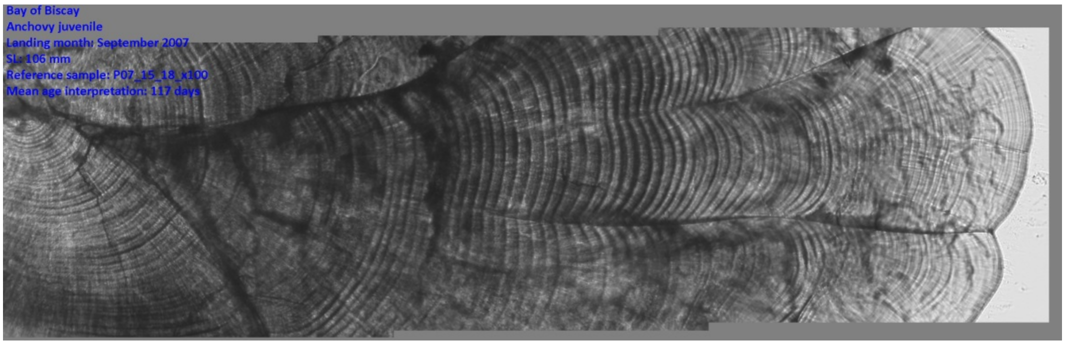
Bay of Biscay

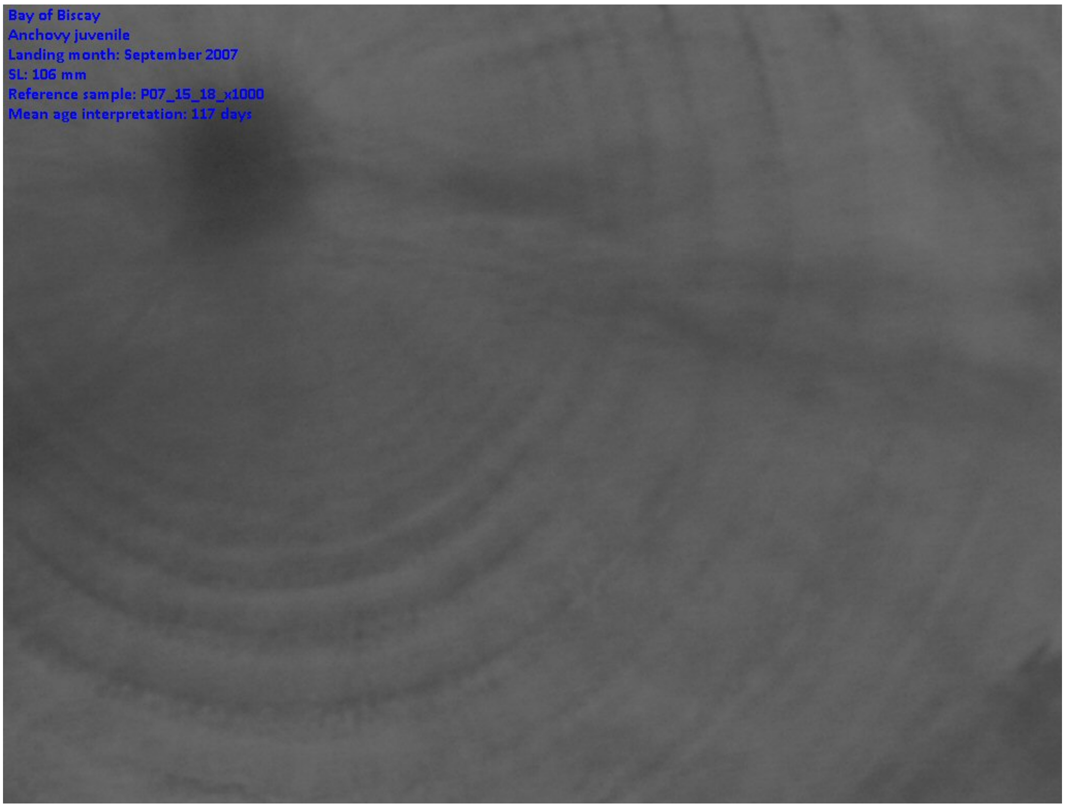




Engraulis encrasicolus juvenile

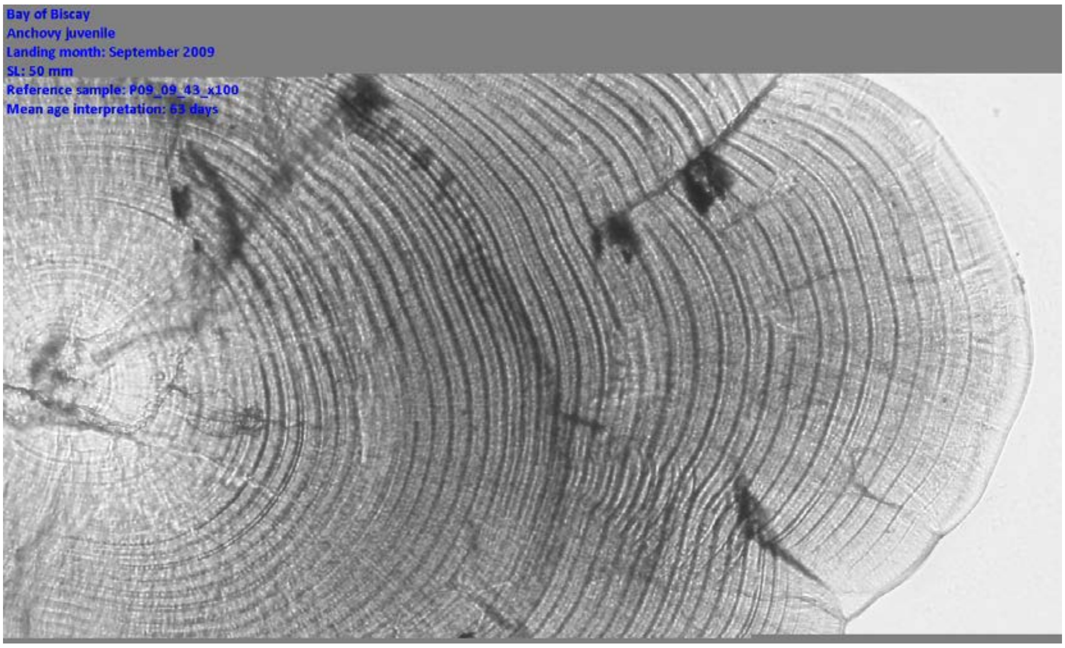
Bay of Biscay

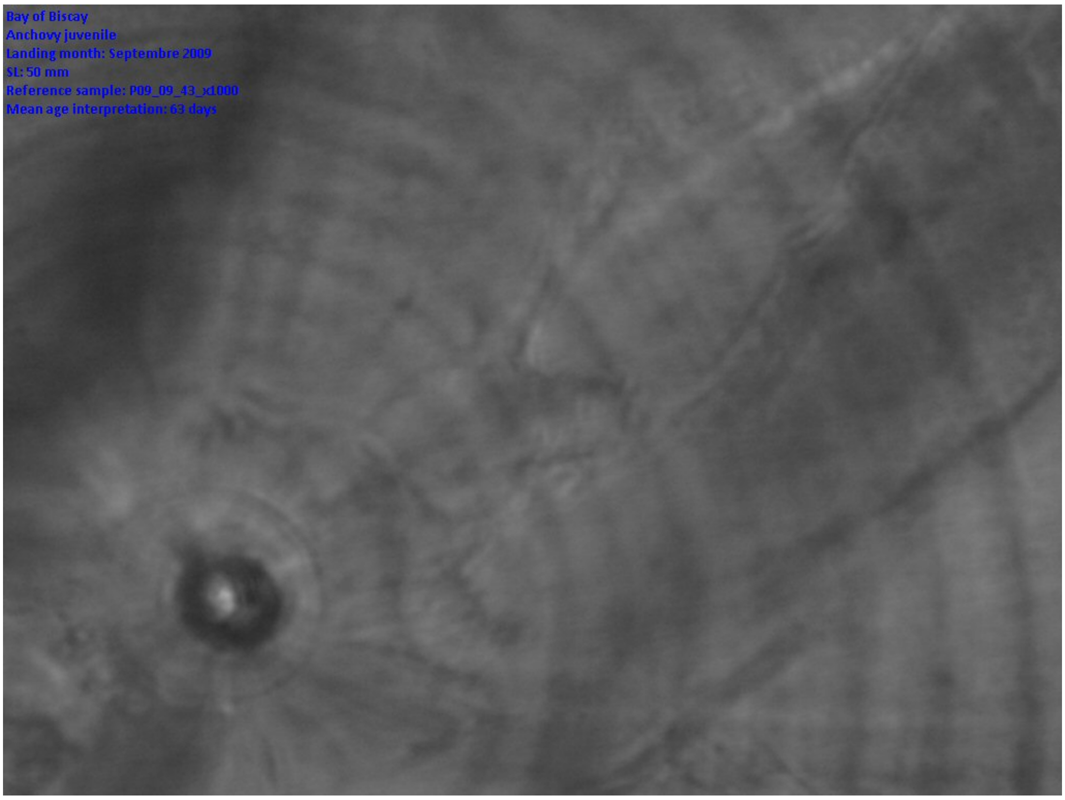




Engraulis encrasicolus juvenile

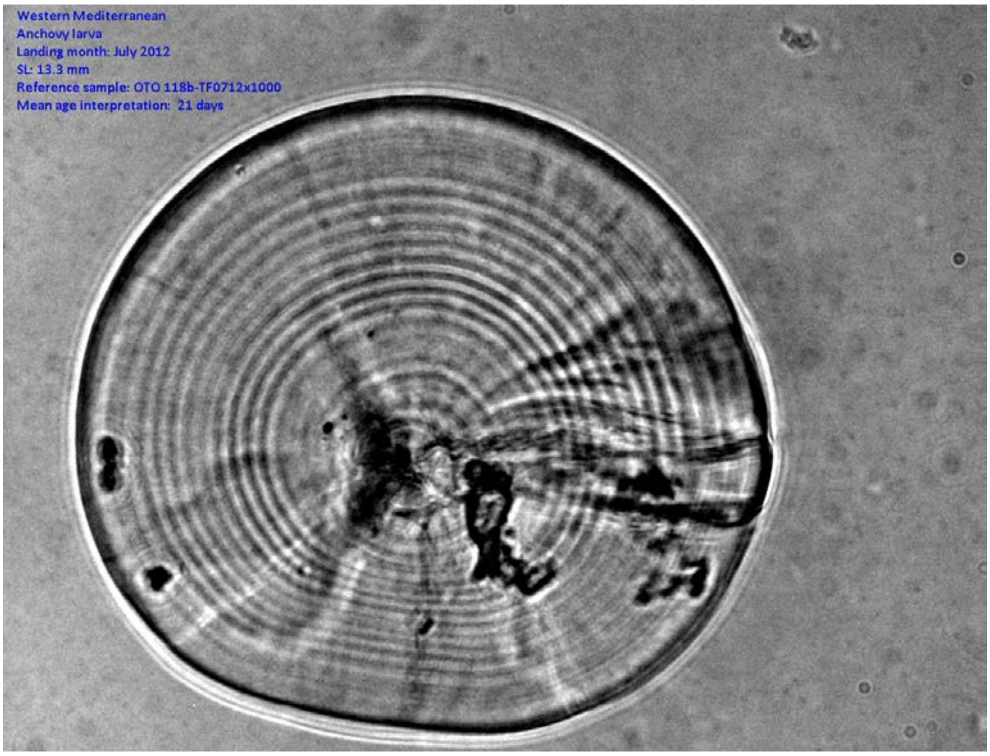
Bay of Biscay





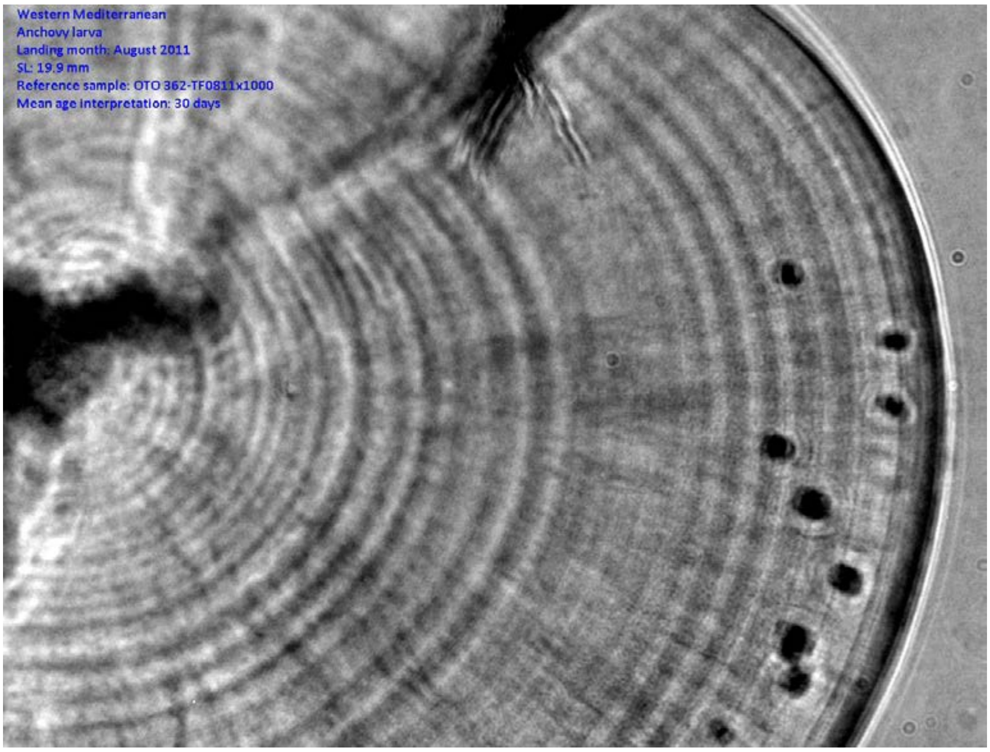
Engraulis encrasicolus larva

Western Mediterranean



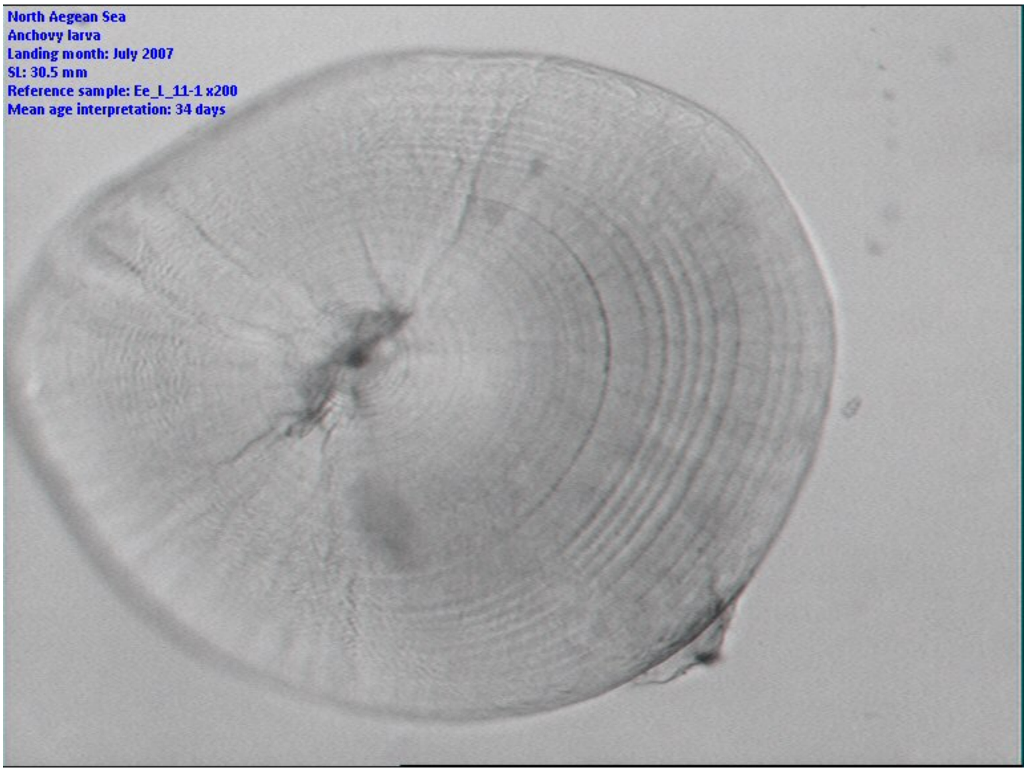
Engraulis encrasicolus larva

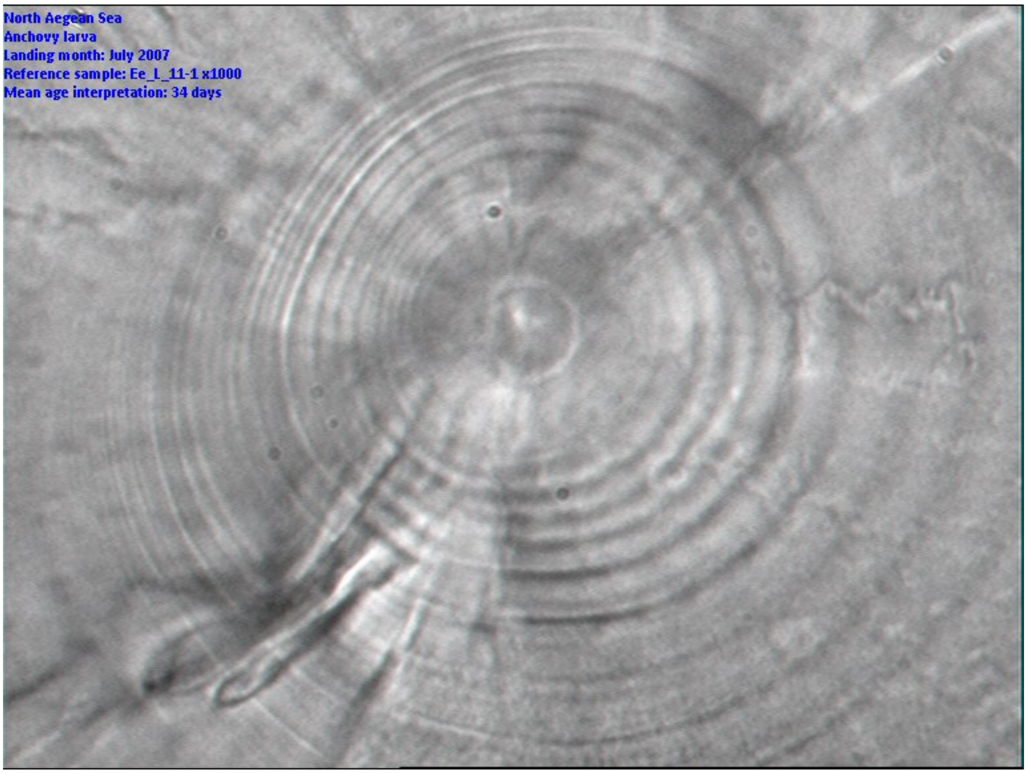
Western Mediterranean



Engraulis encrasicolus larva

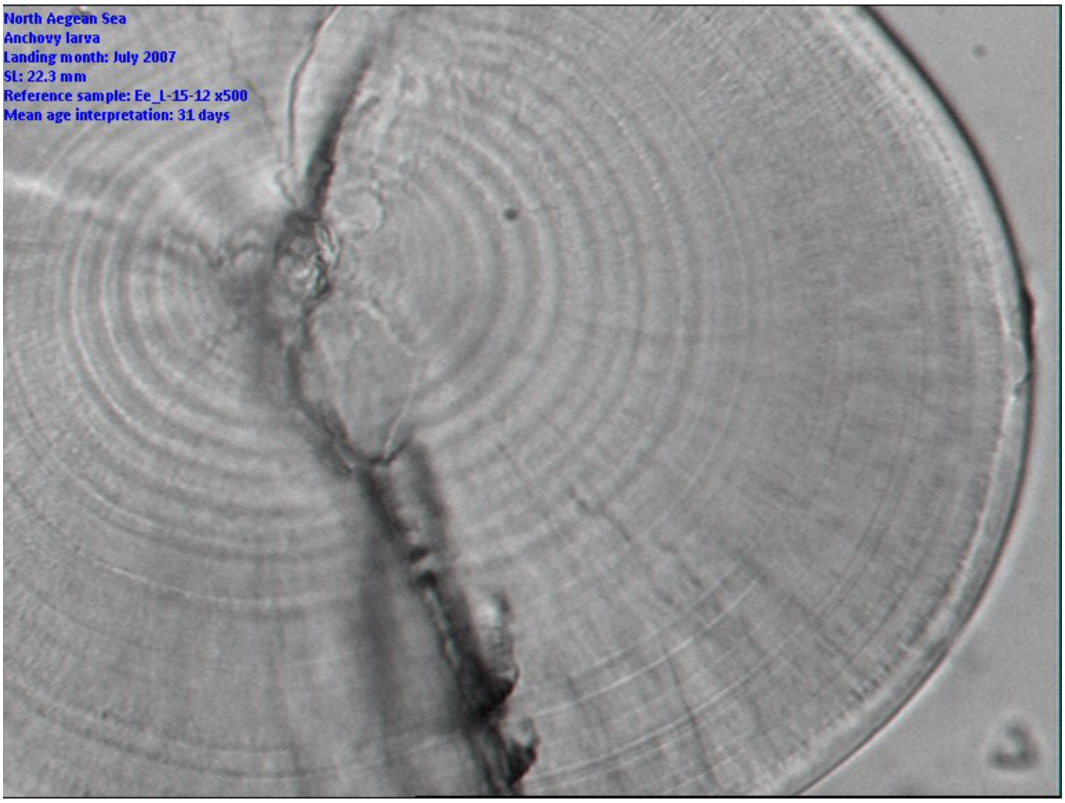
North Aegean Sea

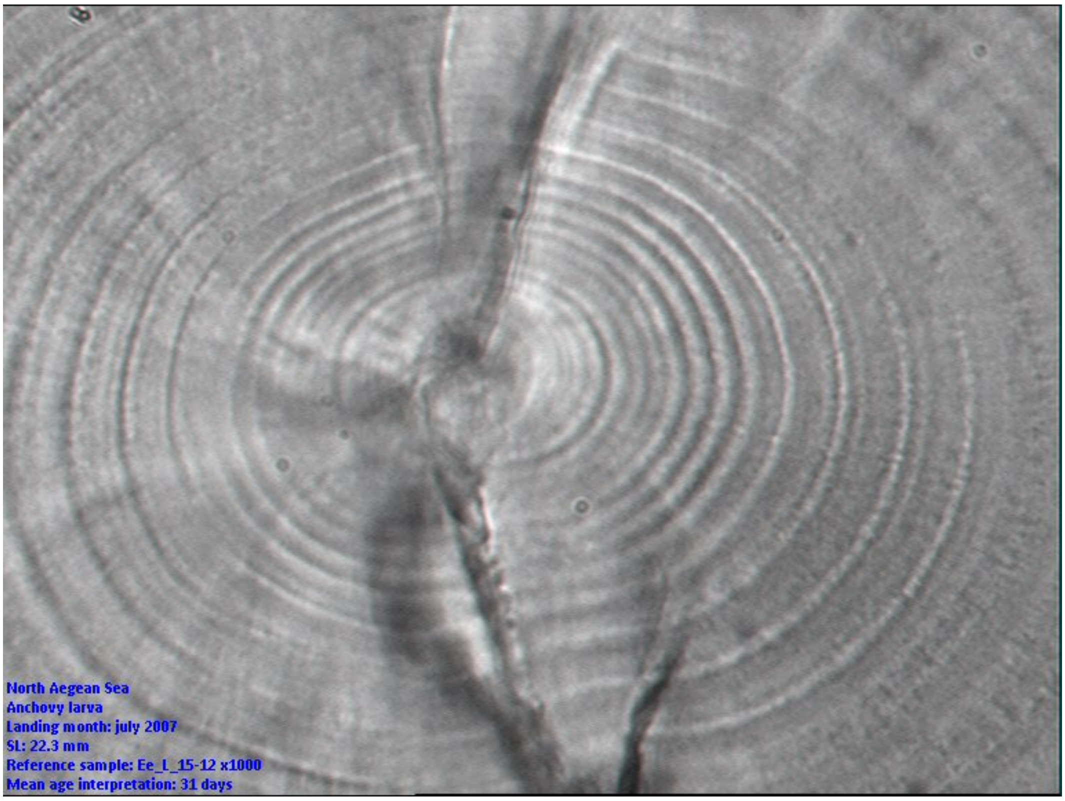




Engraulis encrasicolus larva

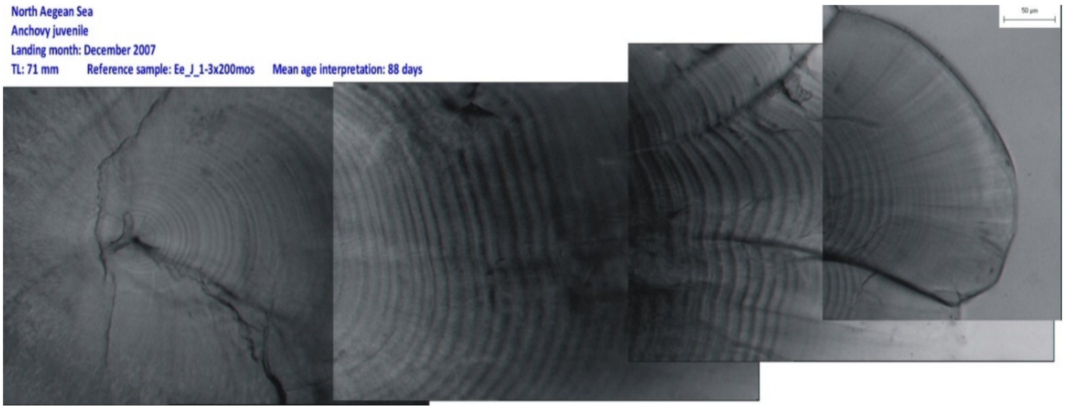
North Aegean Sea

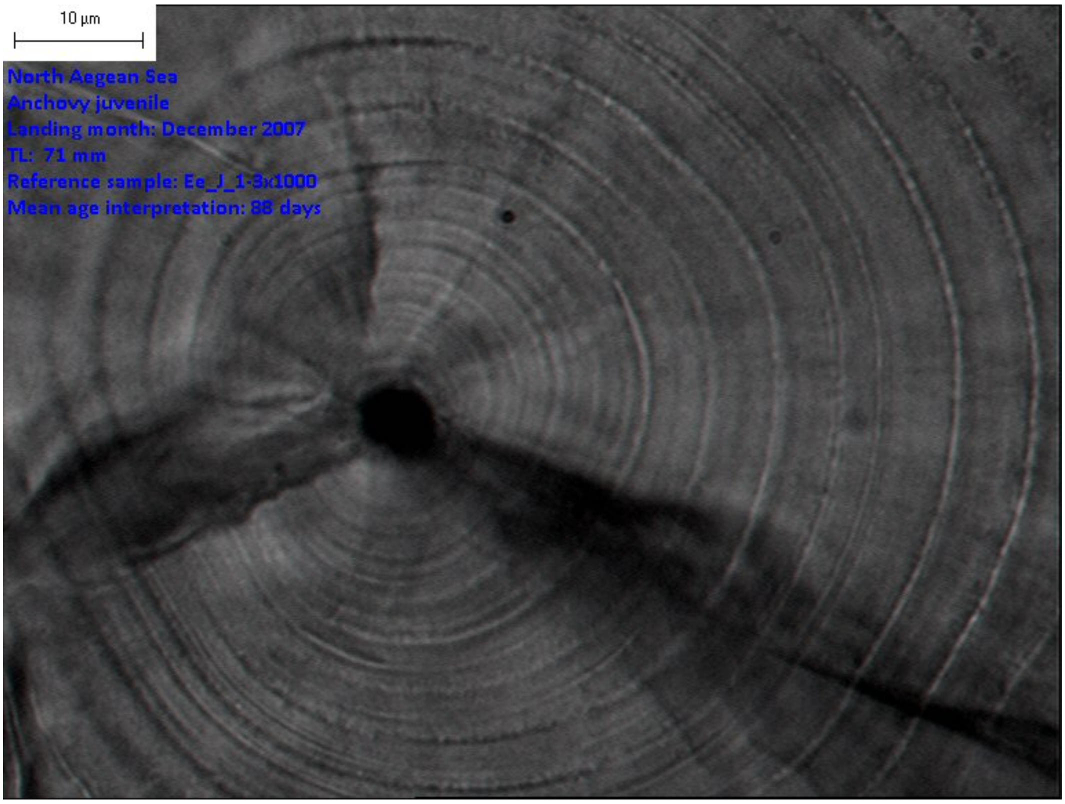




Engraulis encrasicolus juvenile

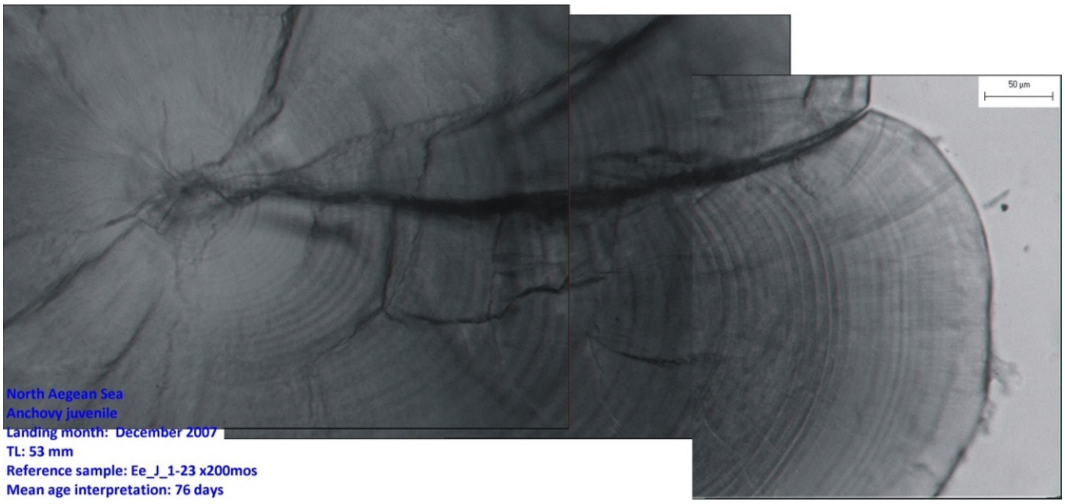
North Aegean Sea

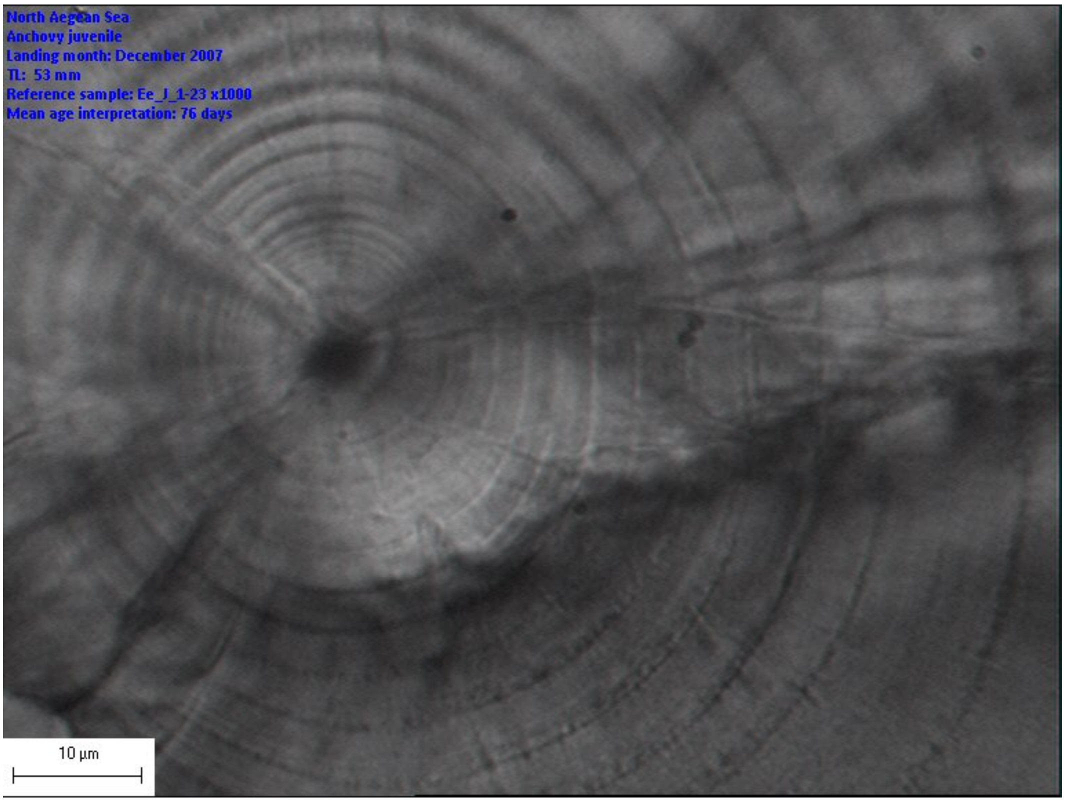




Engraulis encrasicolus juvenile

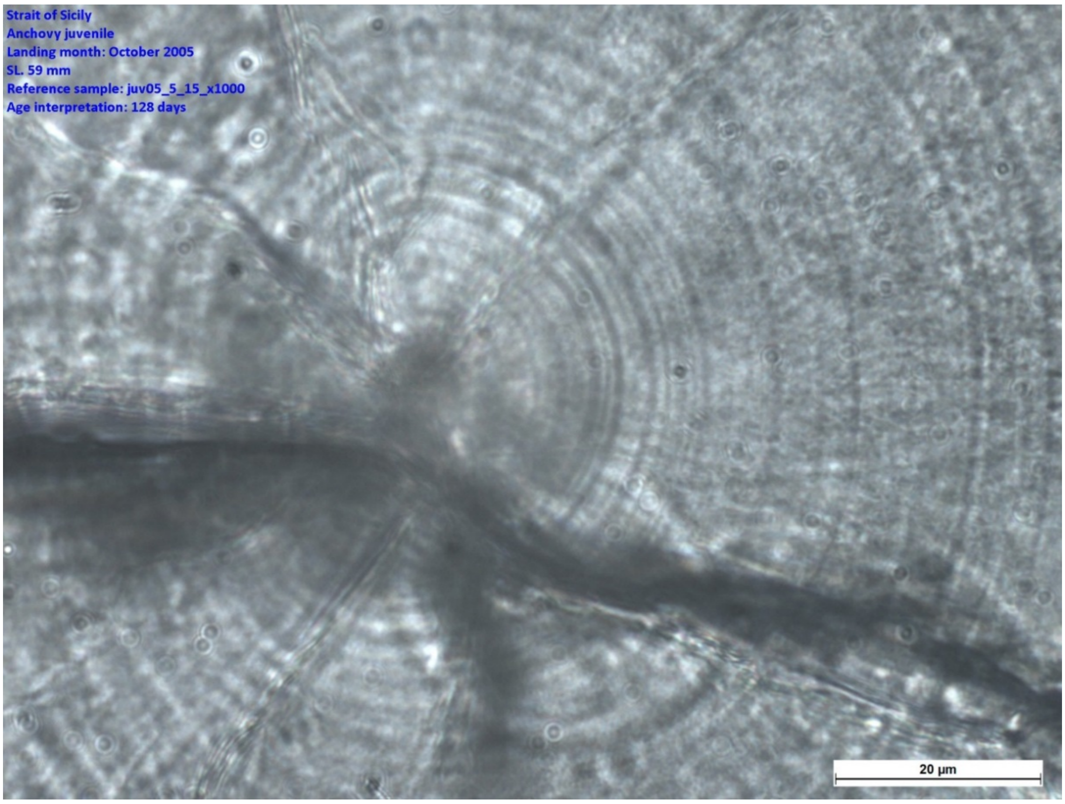
North Aegean Sea





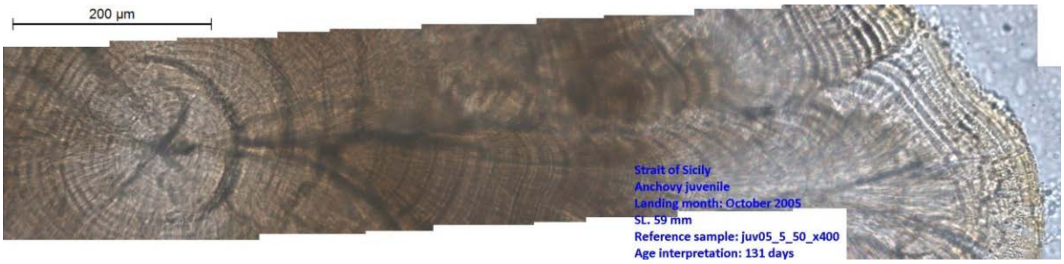
Engraulis encrasicolus juvenile

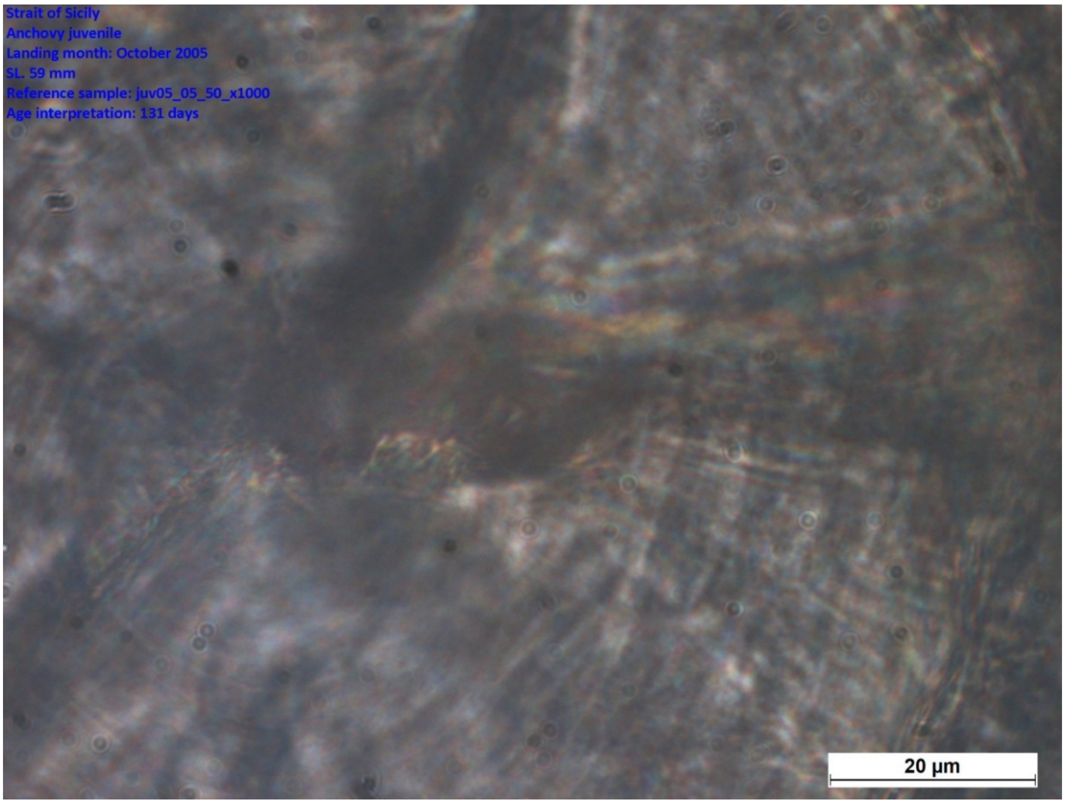
Strait of Sicily



Engraulis encrasicolus juvenile

Strait of Sicily



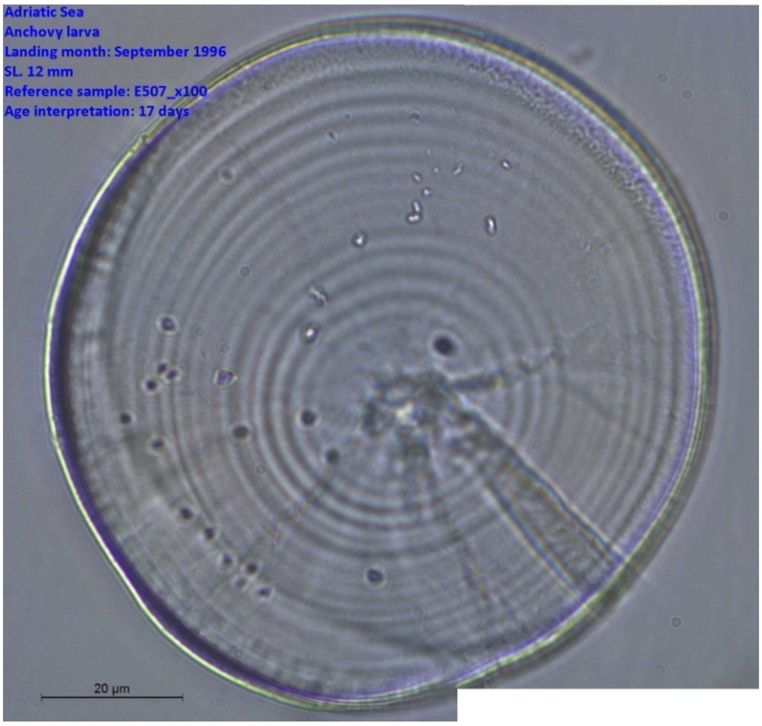


Engraulis encrasicolus larva

Adriatic Sea

Engraulis encrasicolus larva

Adriatic Sea

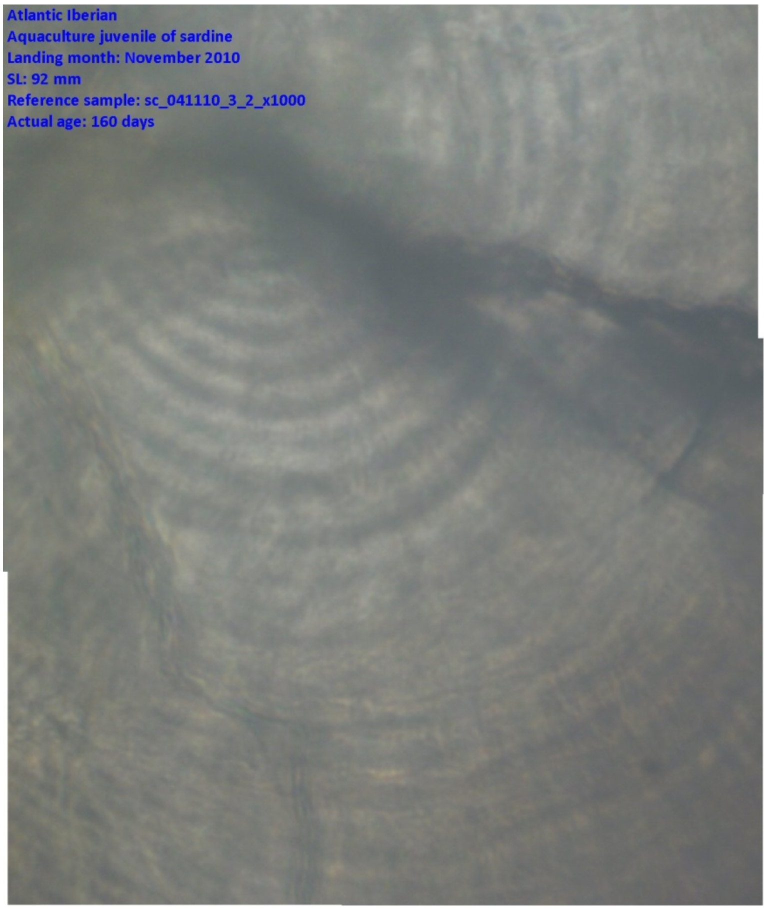


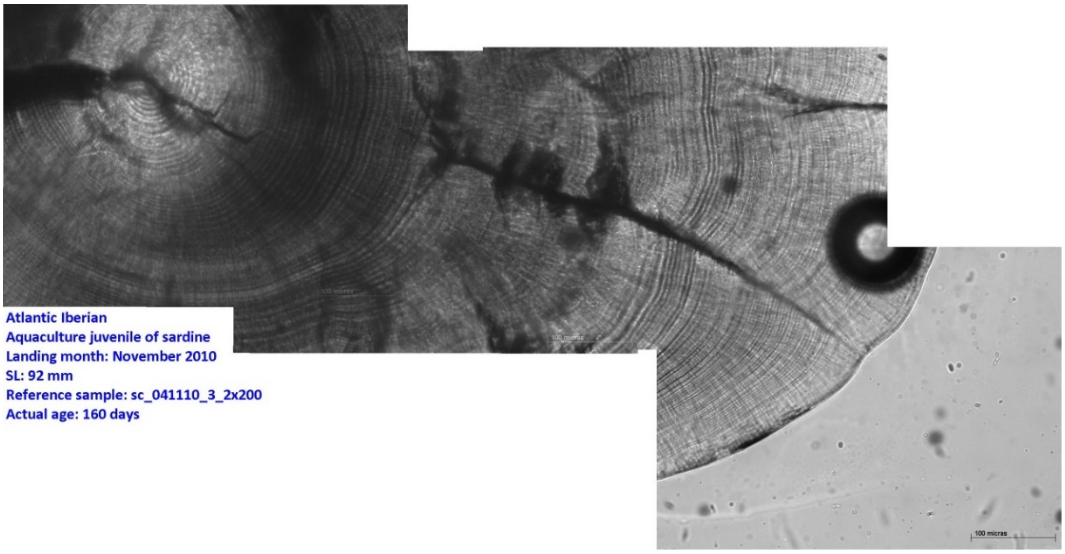
Engraulis encrasicolus juveniles

Adriatic Sea

Sardina pilchardus aquaculture juvenile

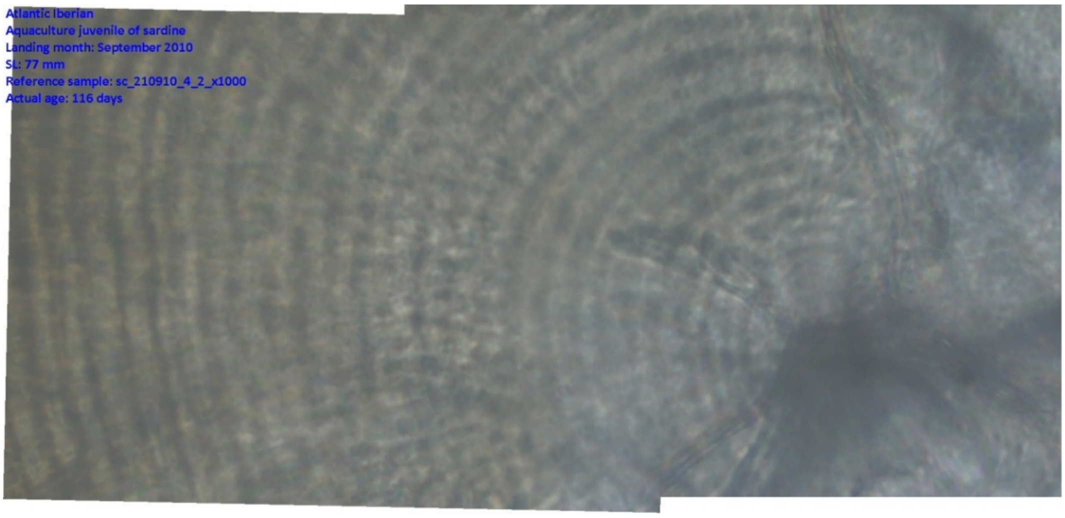
Atlantic Iberian

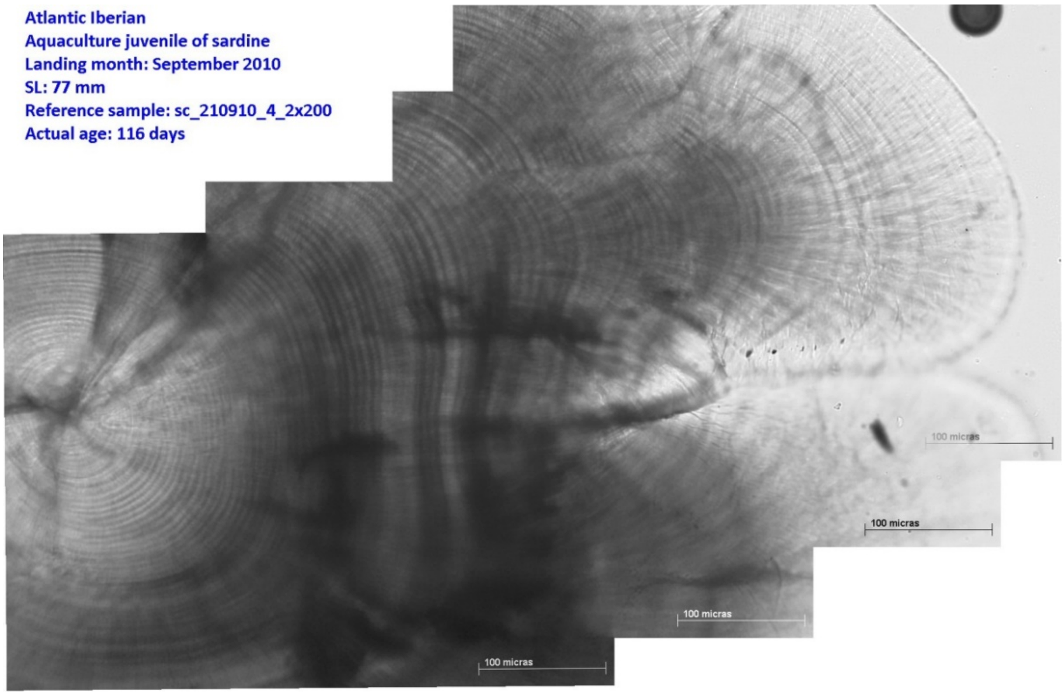




Sardina pilchardus aquaculture juvenile

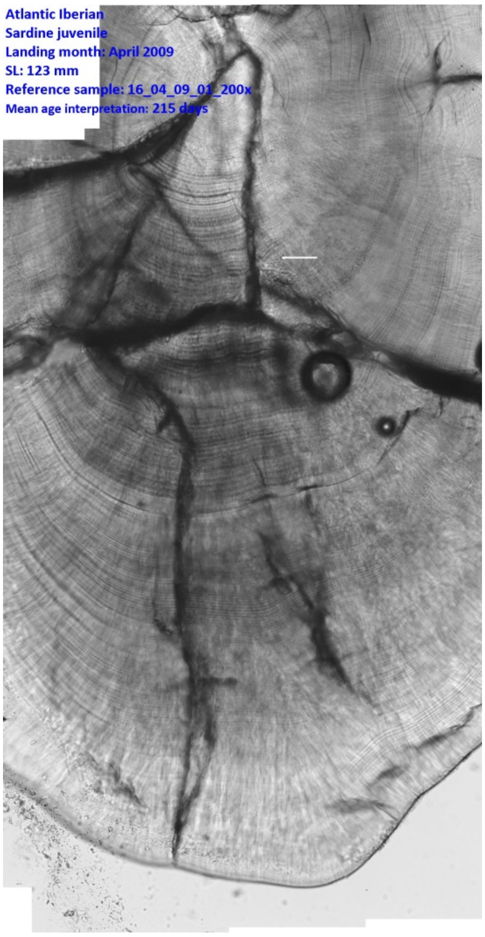
Atlantic Iberian

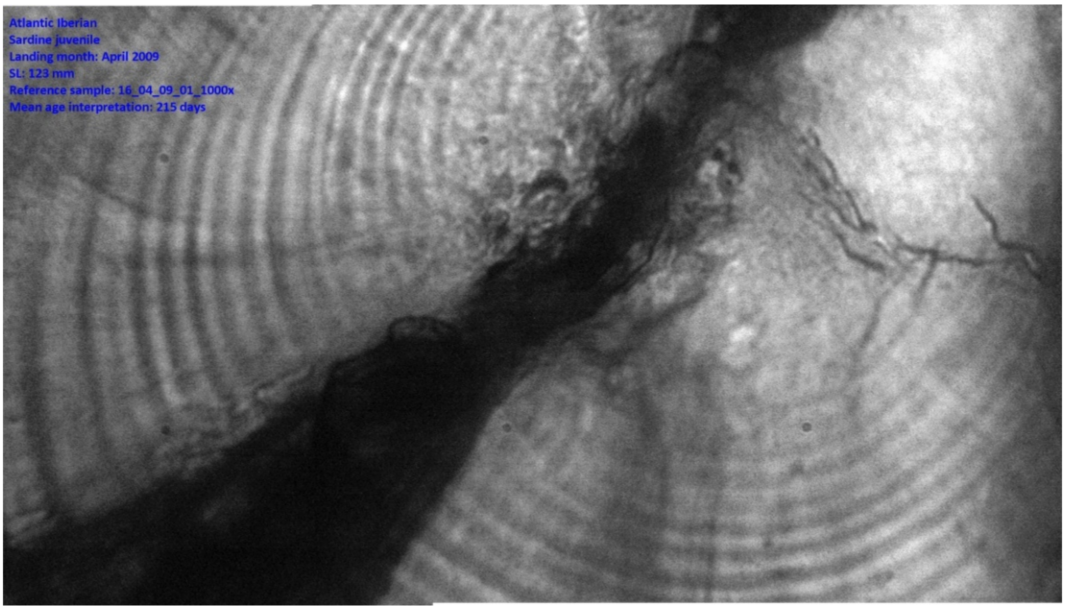




Sardina pilchardus juvenile

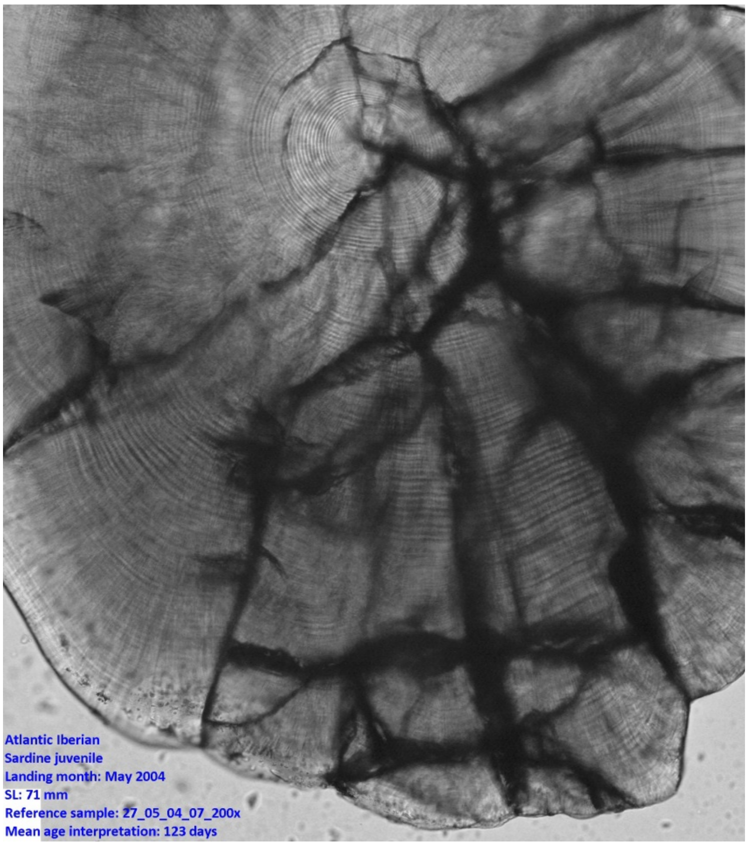
Atlantic Iberian

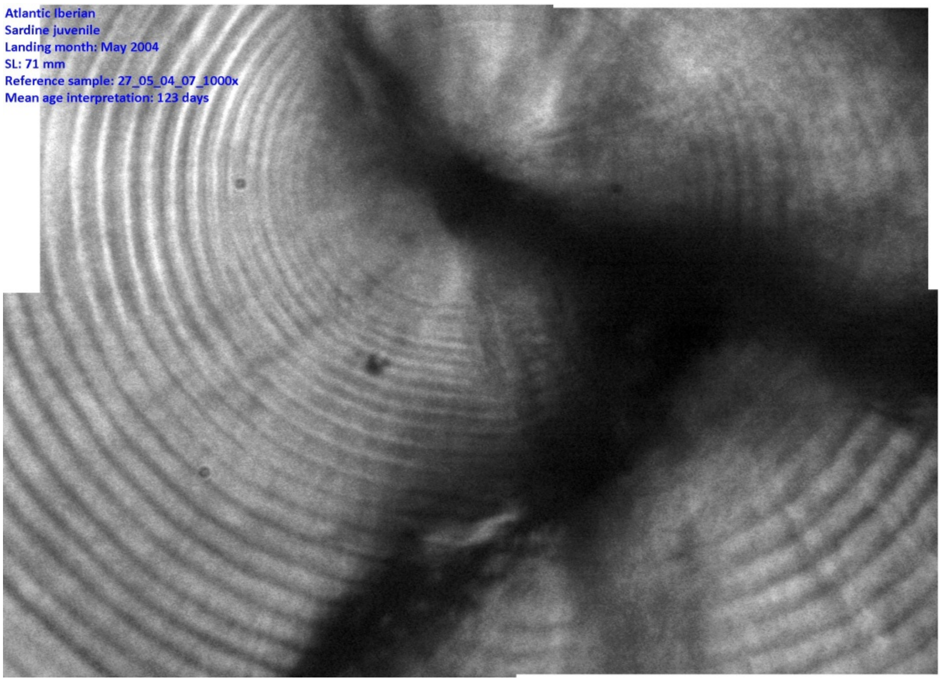




Sardina pilchardus juvenile

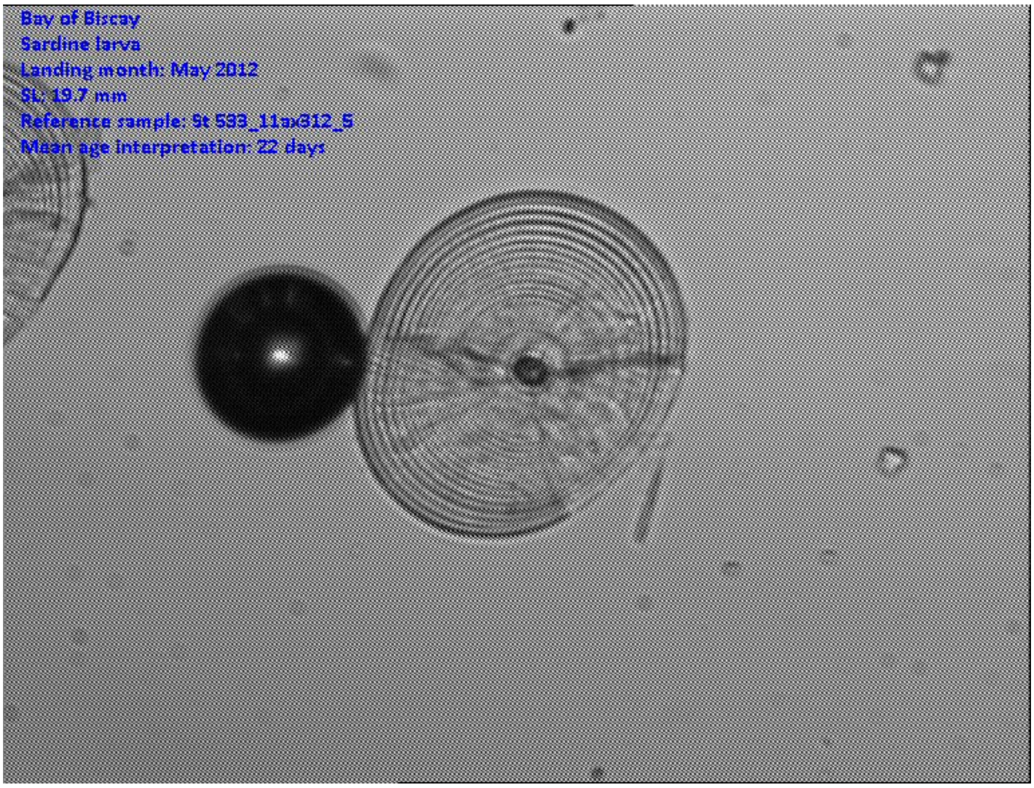
Atlantic Iberian

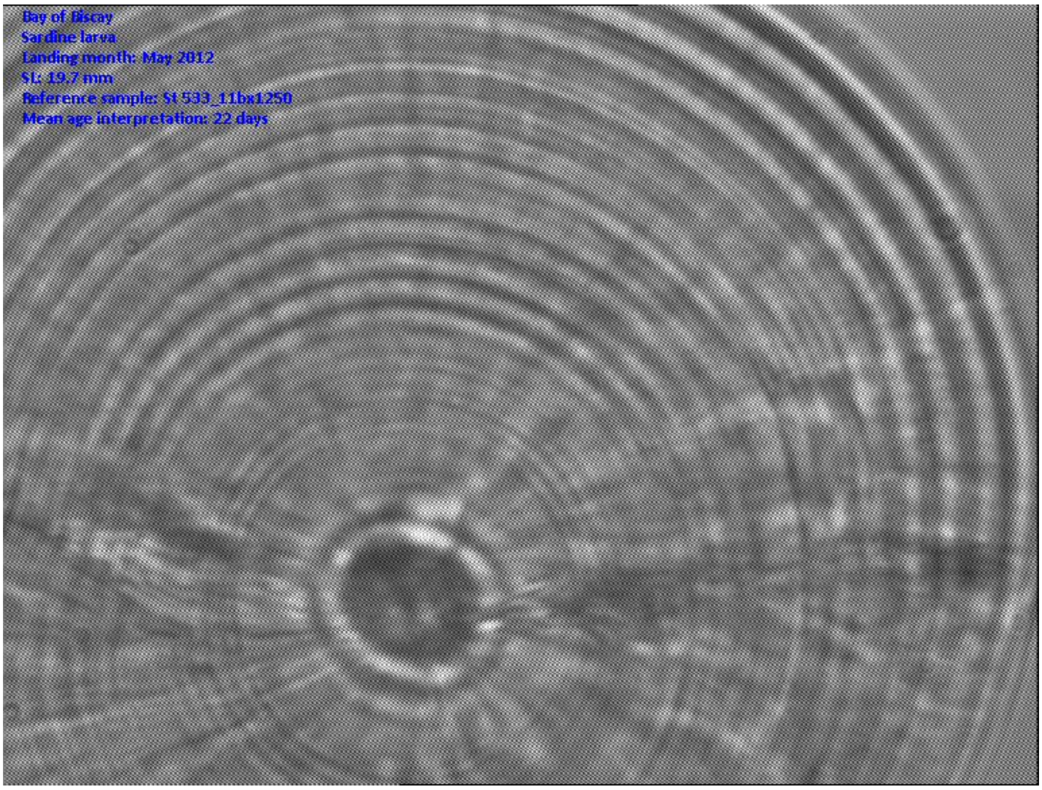




Sardina pilchardus larva

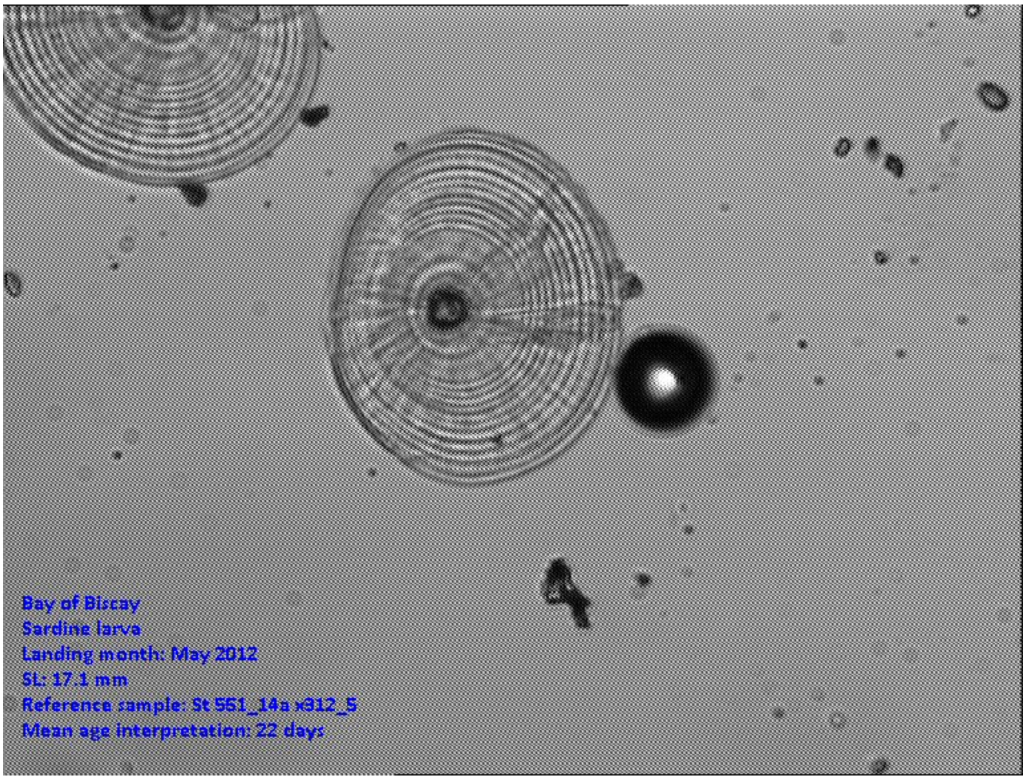
Bay of Biscay

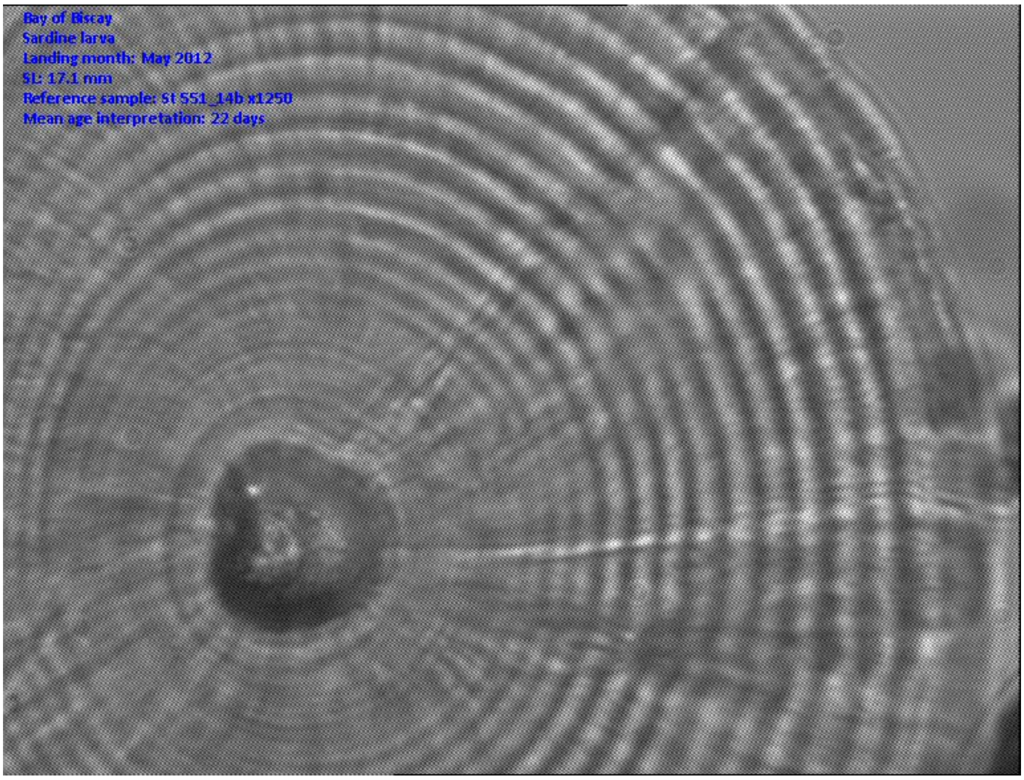




Sardina pilchardus larvae

Bay of Biscay



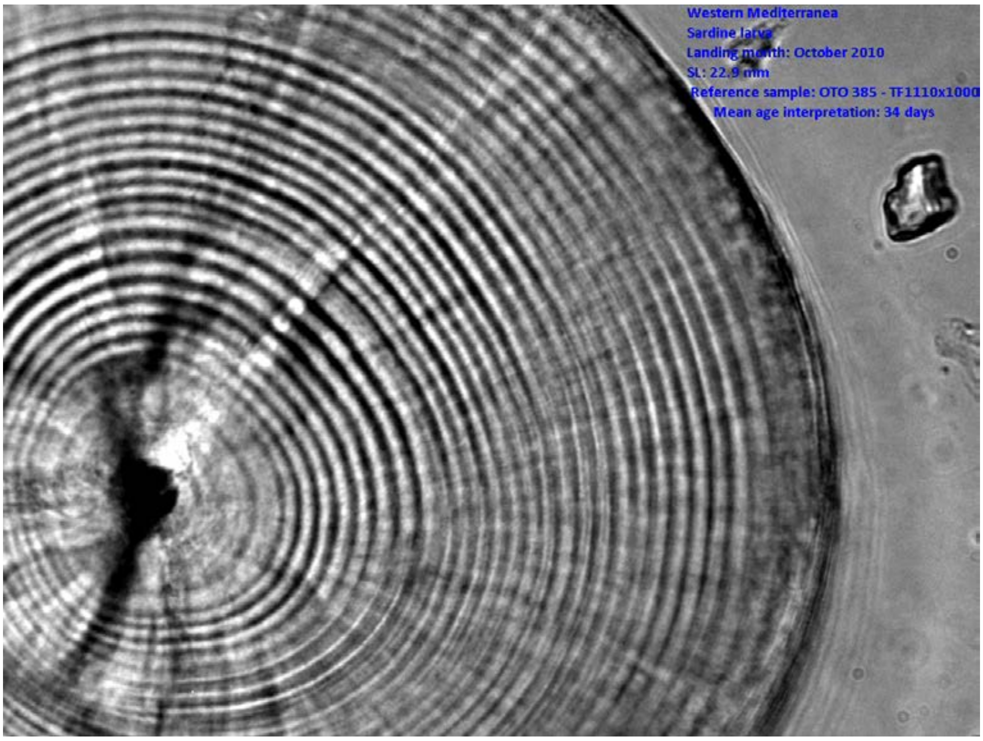


Sardina pilchardus larva

Western Mediterranean

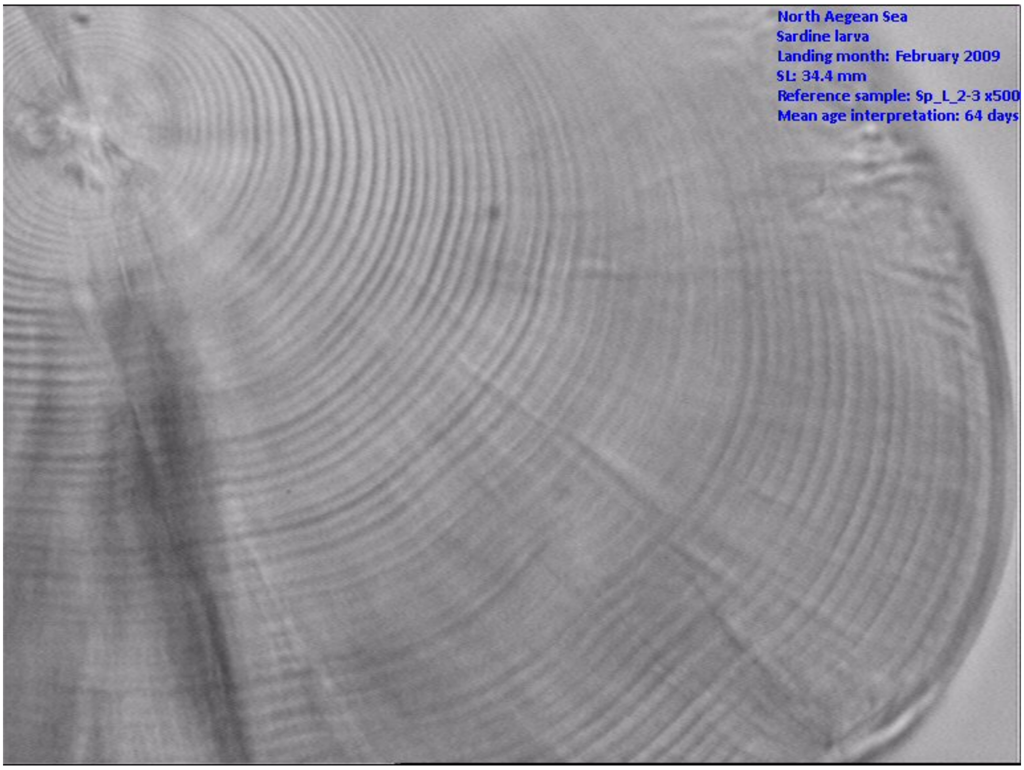
Sardina pilchardus larva

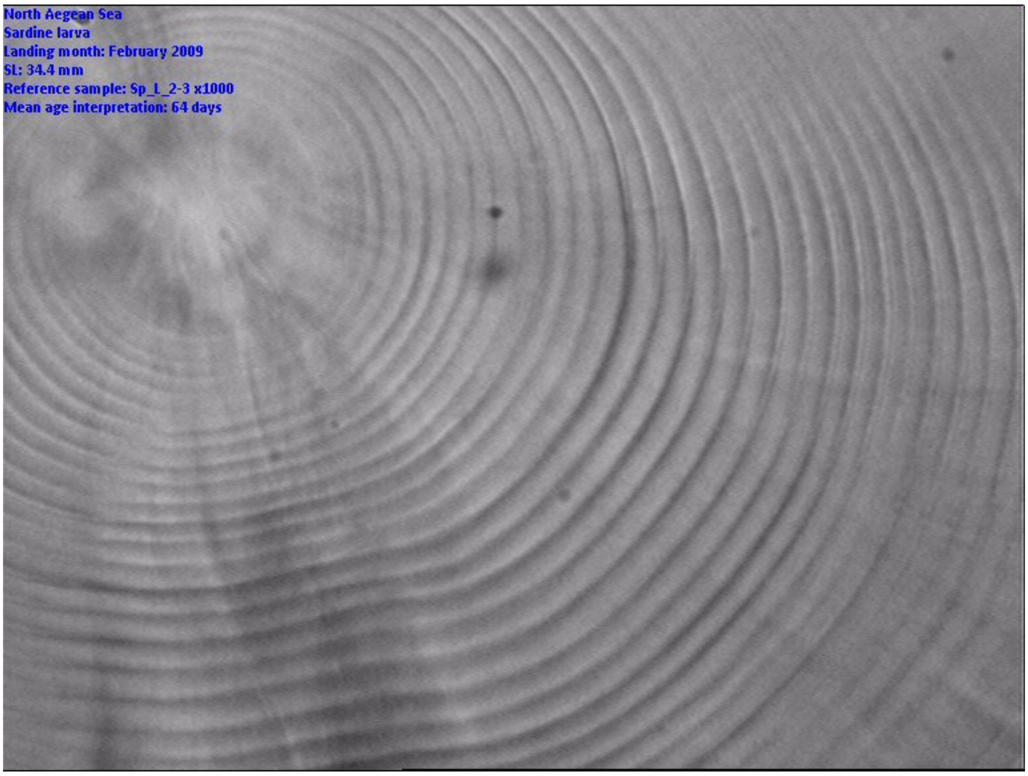
Western Mediterranean



Sardina pilchardus larva

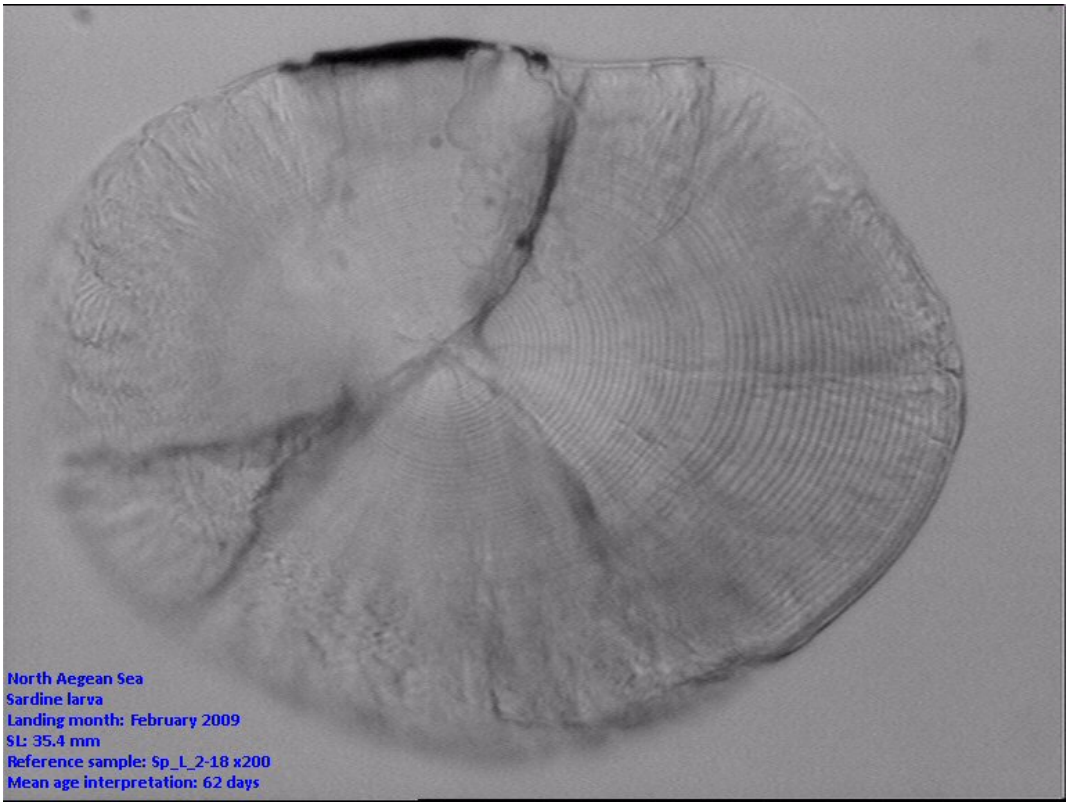
North Aegean Sea

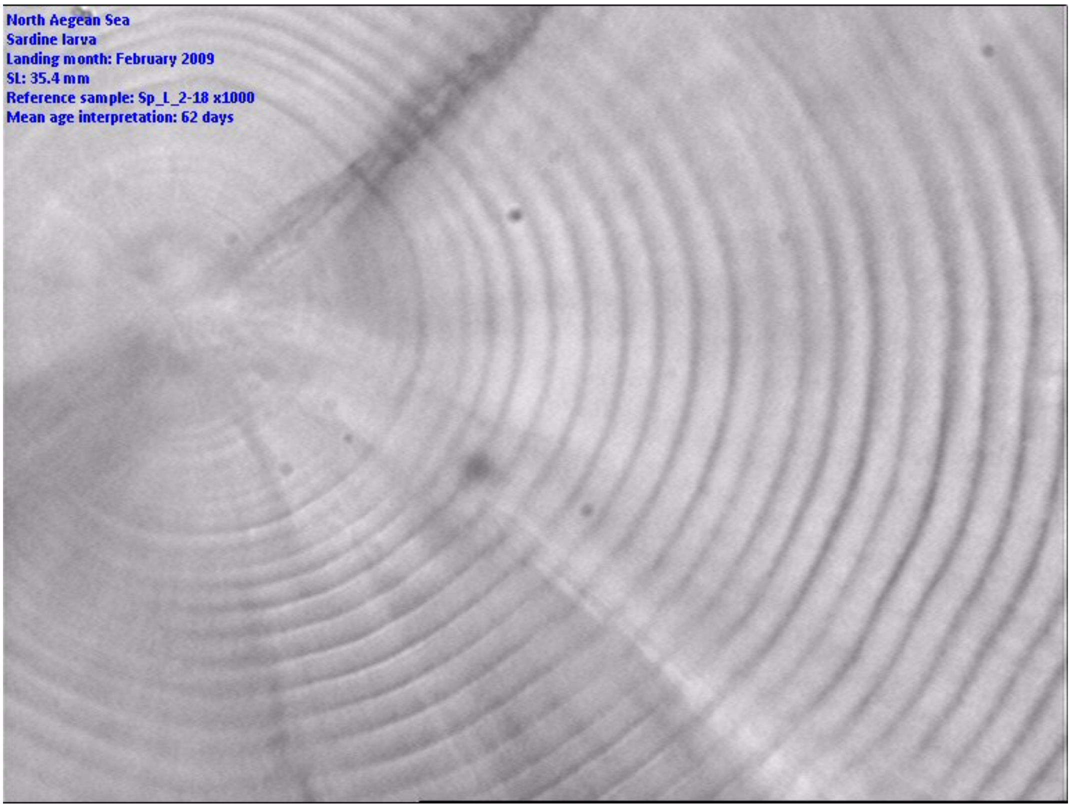




Sardina pilchardus larva

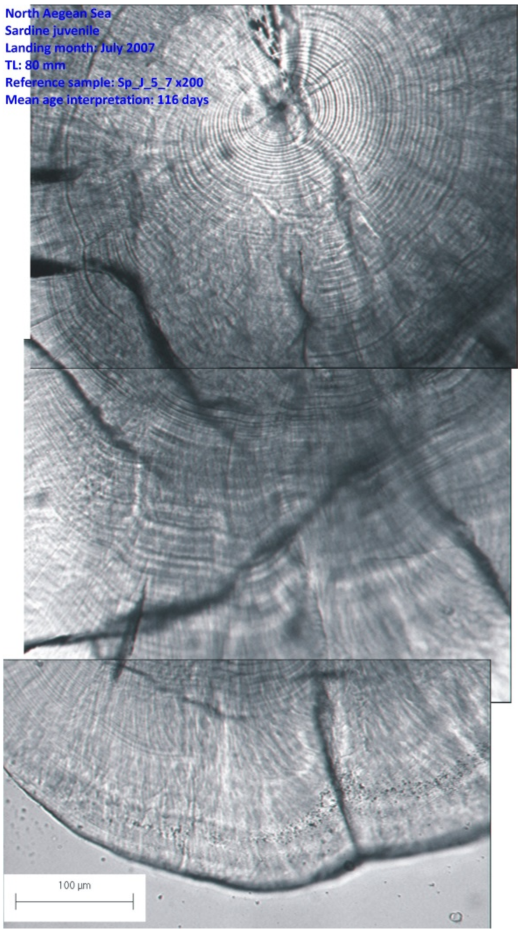
North Aegean Sea

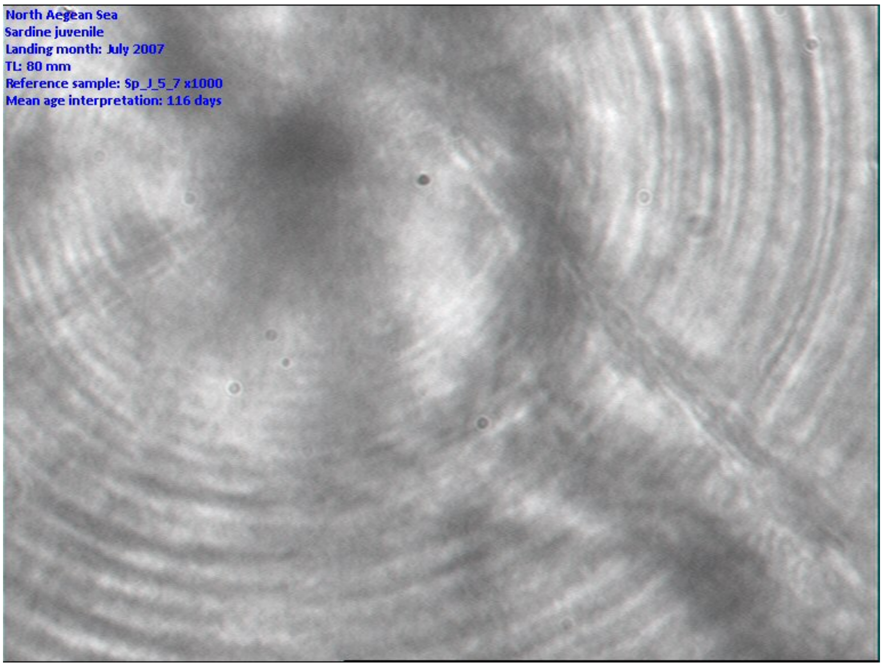




Sardina pilchardus juvenile

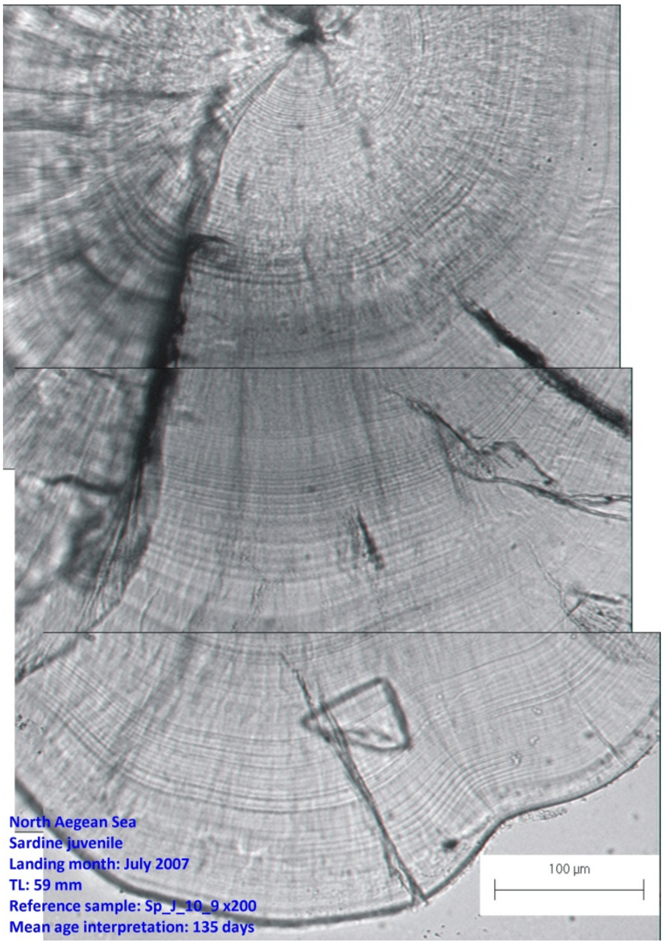
North Aegean Sea

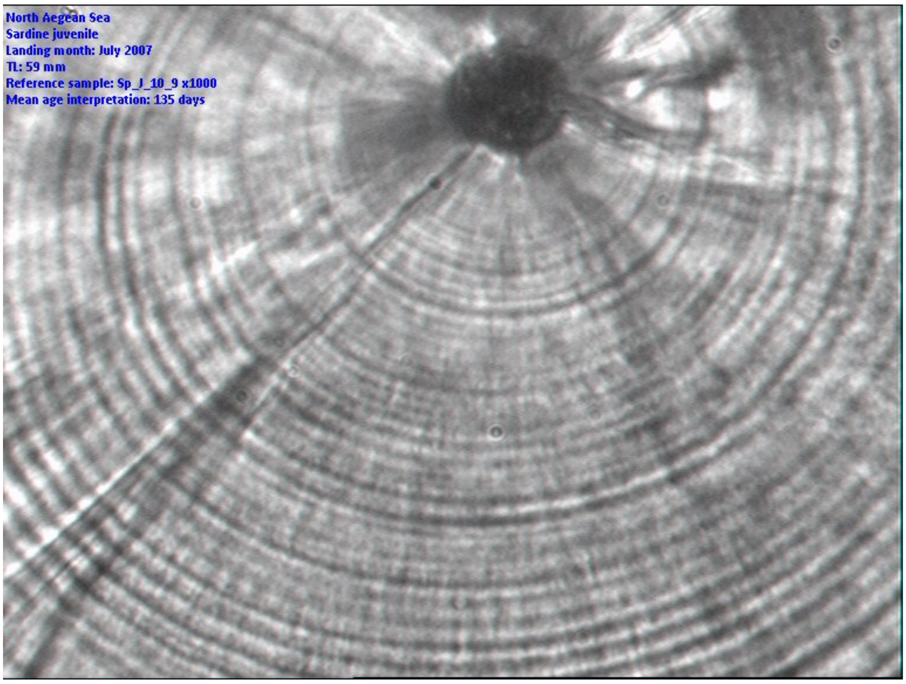




Sardina pilchardus juvenile

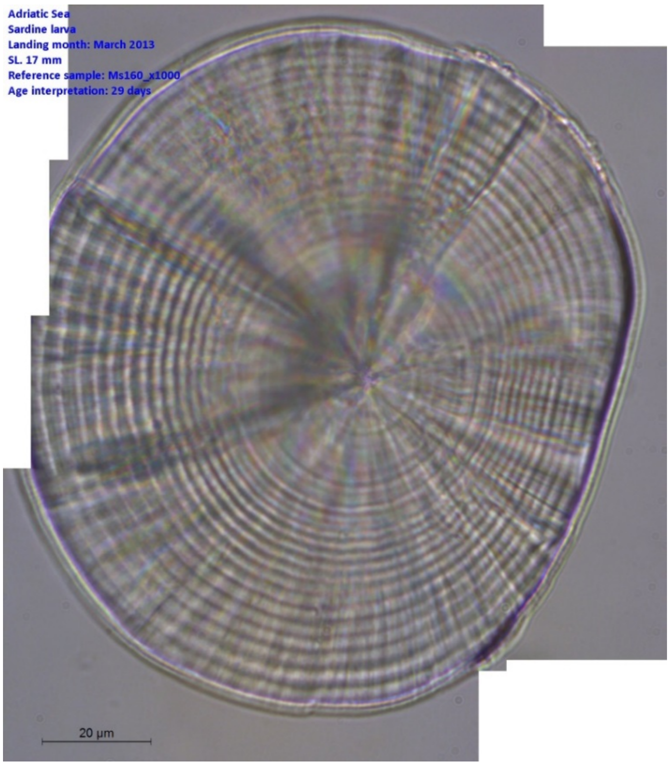
North Aegean Sea





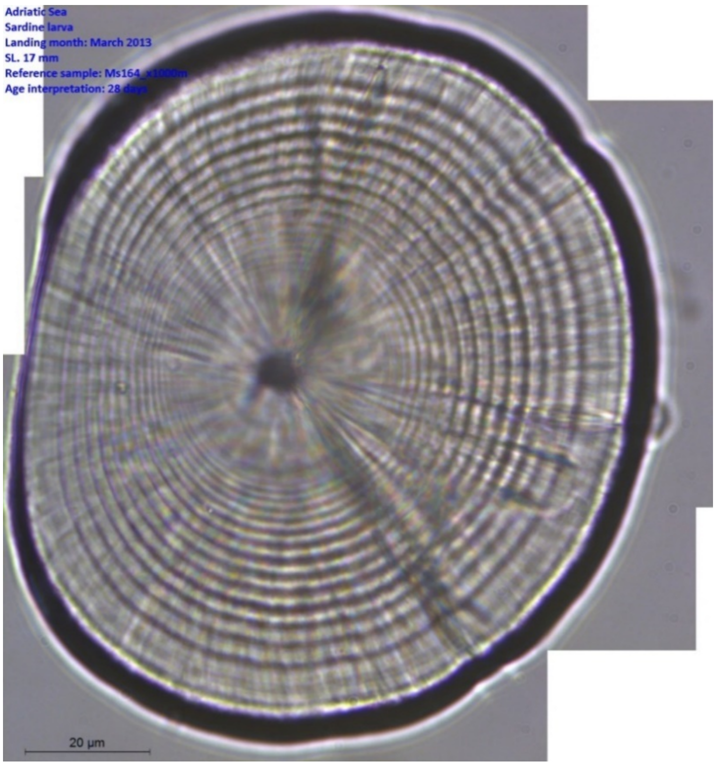
Sardina pilchardus larva

Adriatic Sea



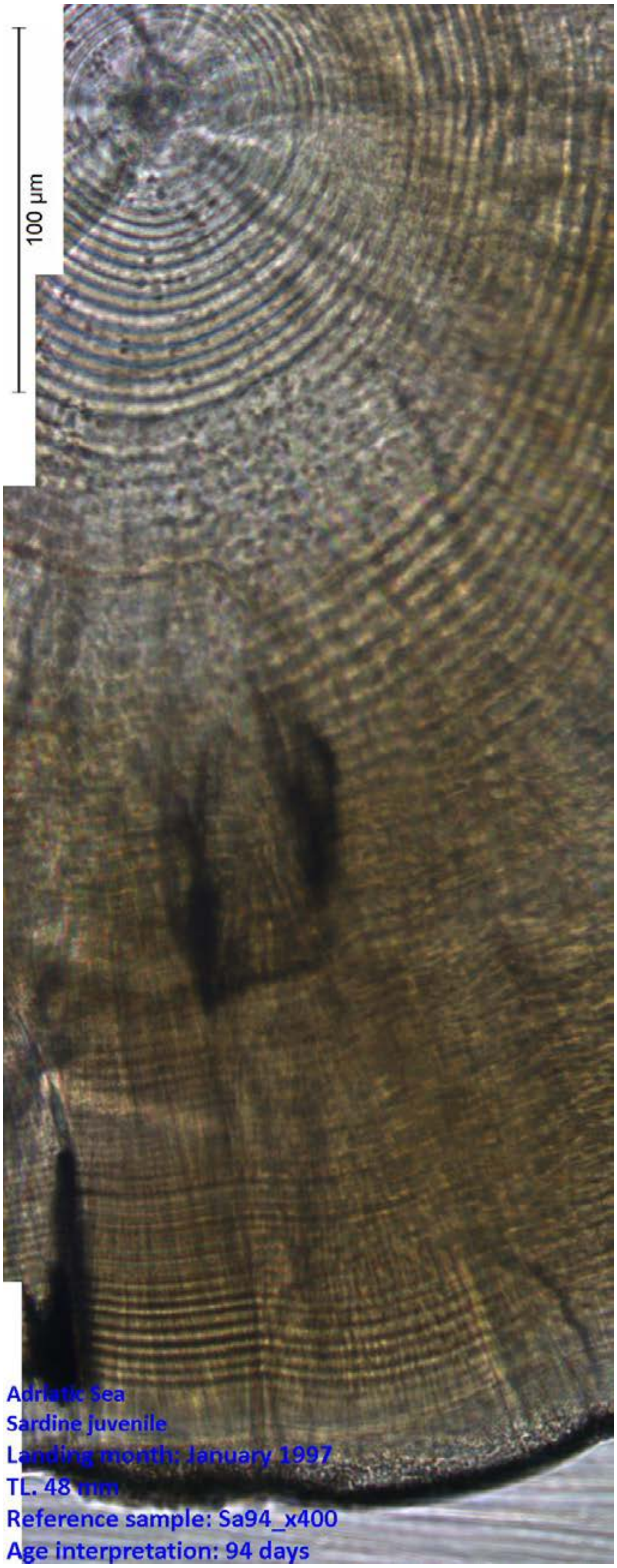
Sardina pilchardus larva

Adriatic Sea



Sardina pilchardus juveniles

Adriatic Sea


100 µm
Adriatic Sea
Sardine juvenile
Landing month: January 1997
TL. 48 mm
Reference sample: Sa94_x400
Age interpretation: 94 days

Annex 6: Results of anchovy otolith reading exercise during WKMIAS

Workshop on Micro increment daily growth in European anchovy and sardine (WKMIAS). Mazara del Vallo, Italy, 21-25 October 2013

Results of Anchovy reading exercise during WKMIAS meeting

On the 22nd October 2013 an inter-calibration exercise of anchovy larvae and juveniles otoliths microstructure analysis was carried out in the laboratory. Nine readers from five institutes with different expertise level were involved in the exercise (see Table 1). The purpose was to reach an agreement on the most suitable magnifications for the reading process and on the ageing interpretation criteria for anchovy daily growth. We used one otolith sample from each area (institute) and produced images at different magnifications.

Table 1. List of participants in the exercise.

Country/Laboratory	Participants in exchange	Expertise level
France/ Ifremer	Elise Bellamy	low
Spain-Basque Country/AZTI	Beatriz Beldarrain	high
Spain-Basque Country/AZTI	Naroa Aldanondo	high
Spain-Atlantic/ IEO (Santander)	Carmen Hernandez	high
Spain-Mediterranean/IEO (Malaga)	Jose Mª Quintanilla	high
Italy-Sicily/ CNR-IAMC	Salvatore Gancitano	high
Italy-Ancona/ CNR-ISMAR	Fortunata Donato	high
Italy-Ancona/ CNR-ISMAR	Monica Panfili	high
Greece/HCMR	Eudoxia Schismenou	high

Anchovy larvae otoliths from Western Mediterranean

The otolith was read at x1000 magnification and all readers agreed that this magnification was the best for anchovy larvae. Daily increment criteria used by all the readers was the Group Band Reading (GBR) (Cermeño *et al.*, 2008).

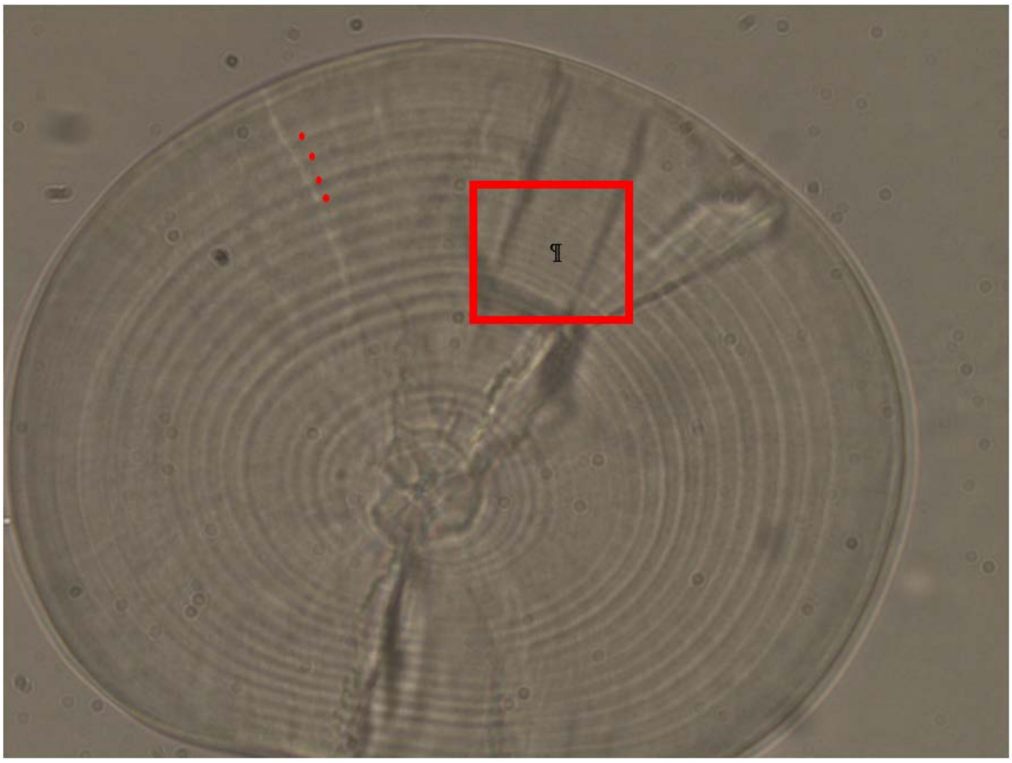


Figure 1: Larval otolith from Western Mediterranean (x1000 magnification), showing growth group band pattern (square).

Anchovy juveniles otoliths from the Bay of Biscay

The otolith was read at x200, x400, x630 magnifications. All the readers agreed on the existence of bands even at higher magnification and decided that GBR method was the most suitable for daily increments counts. Moreover, for all the readers the x200 magnification was the best to distinguish the growth pattern.

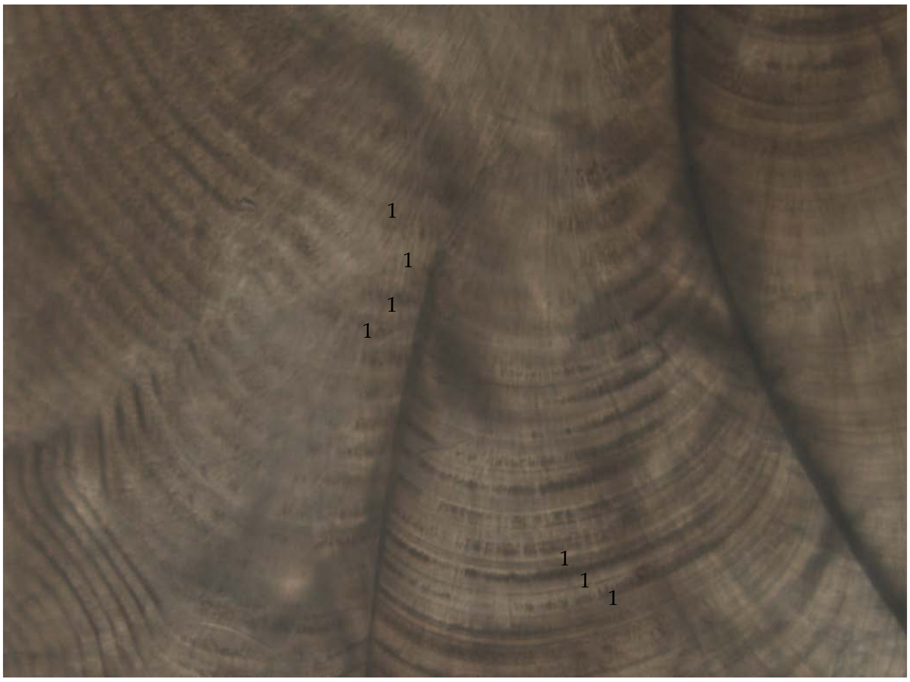


Figure 2: Juvenile otolith from the Bay of Biscay (x200 magnification)

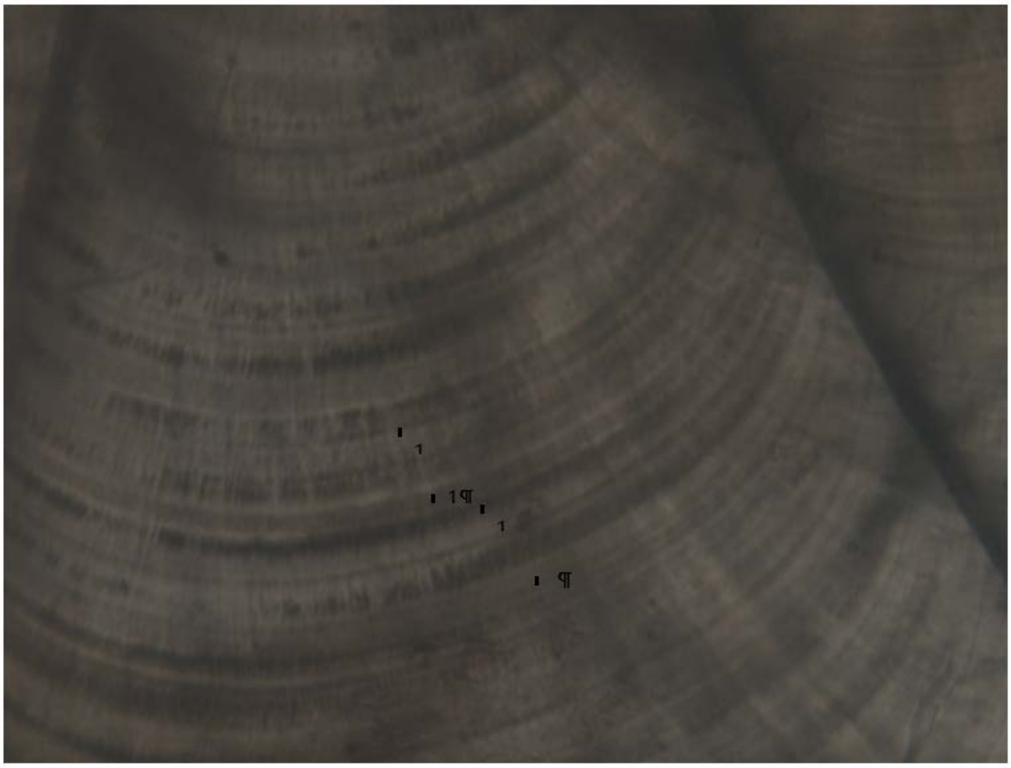


Figure 3: Juvenile otolith from the Bay of Biscay (x400 magnification)

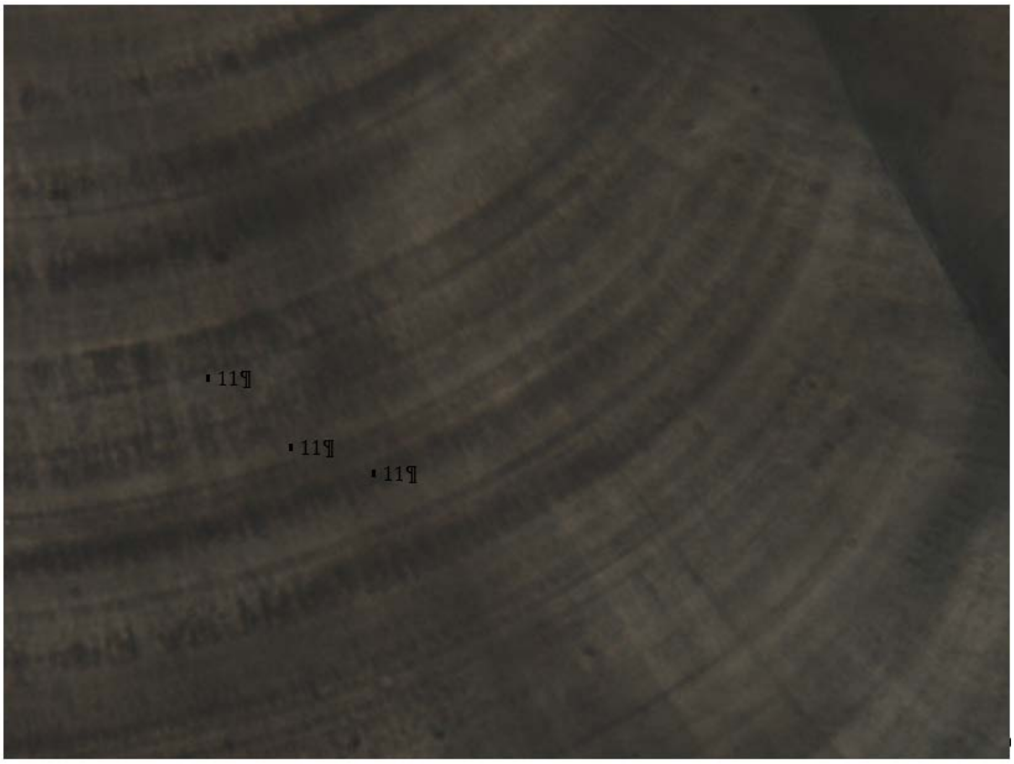


Figure 4: Juvenile otolith from the Bay of Biscay (x630 magnification)

Anchovy juveniles otoliths from the Adriatic Sea

The otolith was read at x200, x400, x630 magnifications. For 67% of the readers the x200 magnification was the best, while 33% preferred the x400 magnification. The ageing interpretation criteria were different among the readers: 67% of the readers applied the GBR (**B** in Figures 5, 6 and 7) and the 33% applied the IMR (**I** in Figures 5,6 and 7). This resulted in different increment counts in the selected area of the otolith, i.e, IMR criteria doubled the number of increment counts compared with GBR method at any magnification.

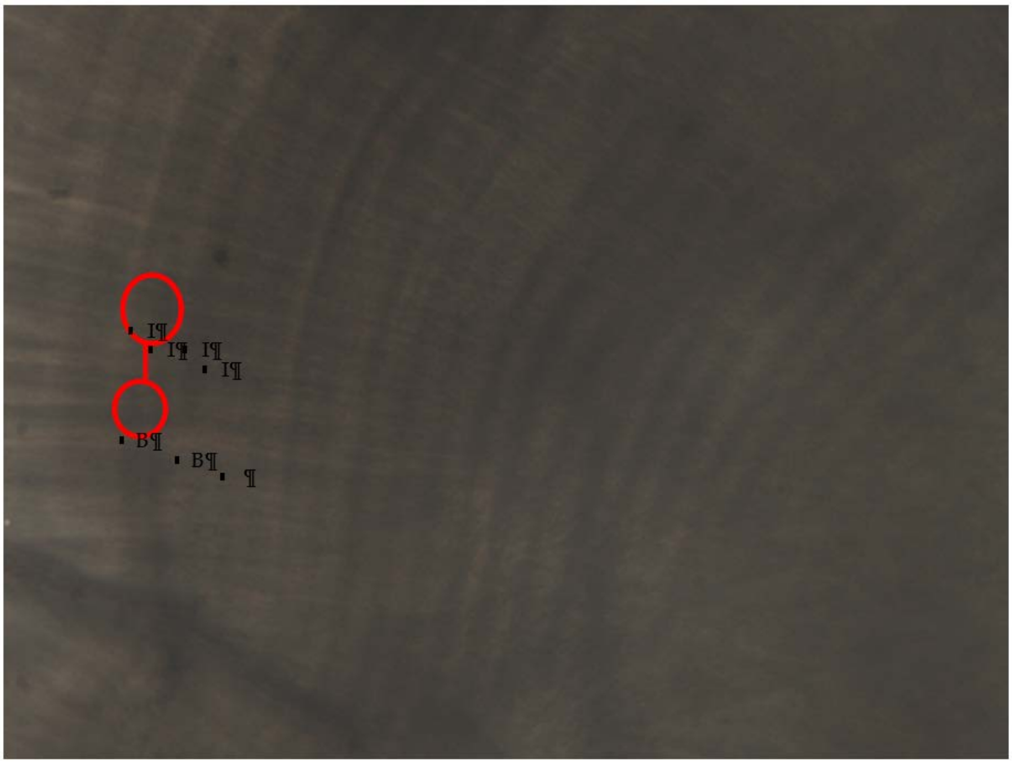


Figure 5: Juvenile otolith from the Adriatic Sea (x200 magnification). B and I mark GBR and IMR increment counts, respectively.

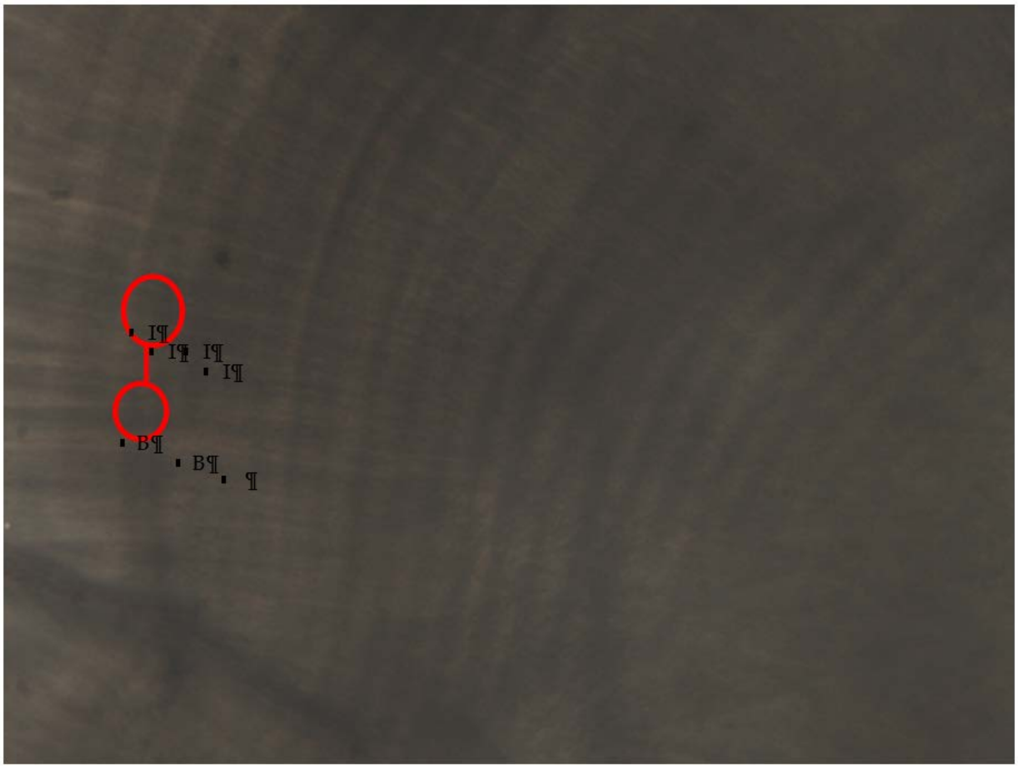


Figure 6: Juvenile otolith from the Adriatic Sea (x400 magnification). B and I mark GBR and IMR increment counts, respectively.

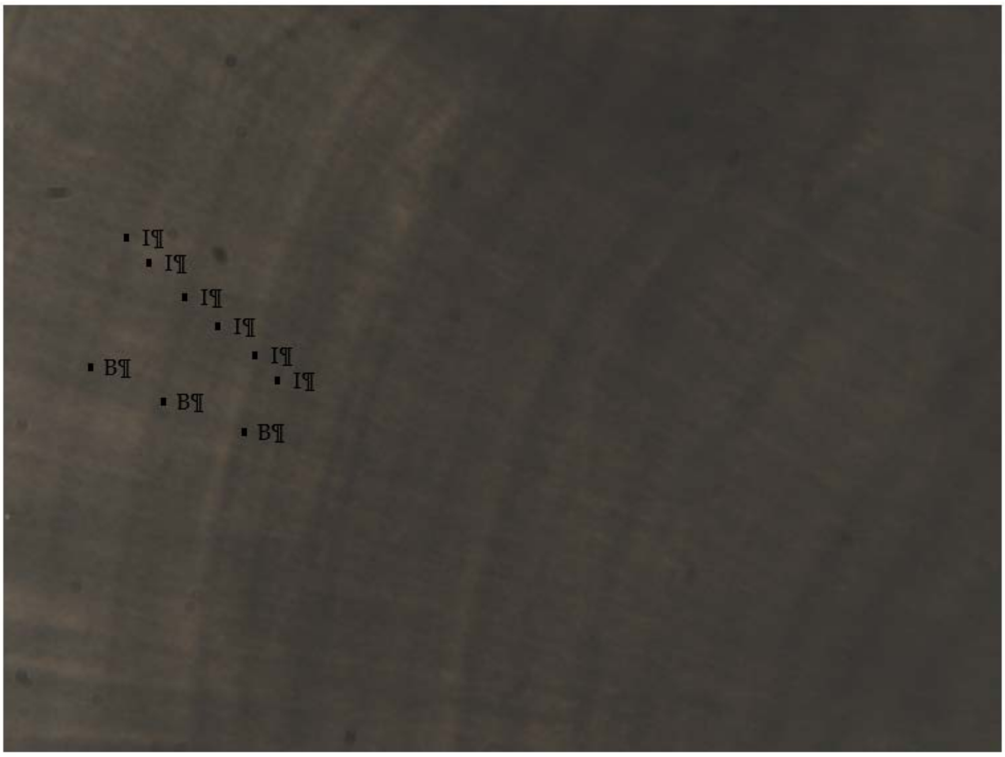


Figure 7: Juvenile otolith from the Adriatic Sea (x630 magnification). B and I mark GBR and IMR increment counts, respectively.

Anchovy juveniles otoliths from North Aegean Sea

The otolith was read at x200, x400, x630 magnifications. Moreover, in this case for each magnification we obtained two images differently focused (Focus 1 in Figures 8, 10 and 12 and Focus 2 in Figures 9, 11 and 13). At every magnification, all readers agreed on the interpretation of the daily growth pattern following the GBR criteria, even when a double pattern was visible (Focus 2 in Figures 9, 11 and 13). In addition, all readers agreed that the x200 magnification was the best because in higher magnifications (x400, x630) the appearance of subdaily rings made more difficult the reading process and could cause overestimation of the age.

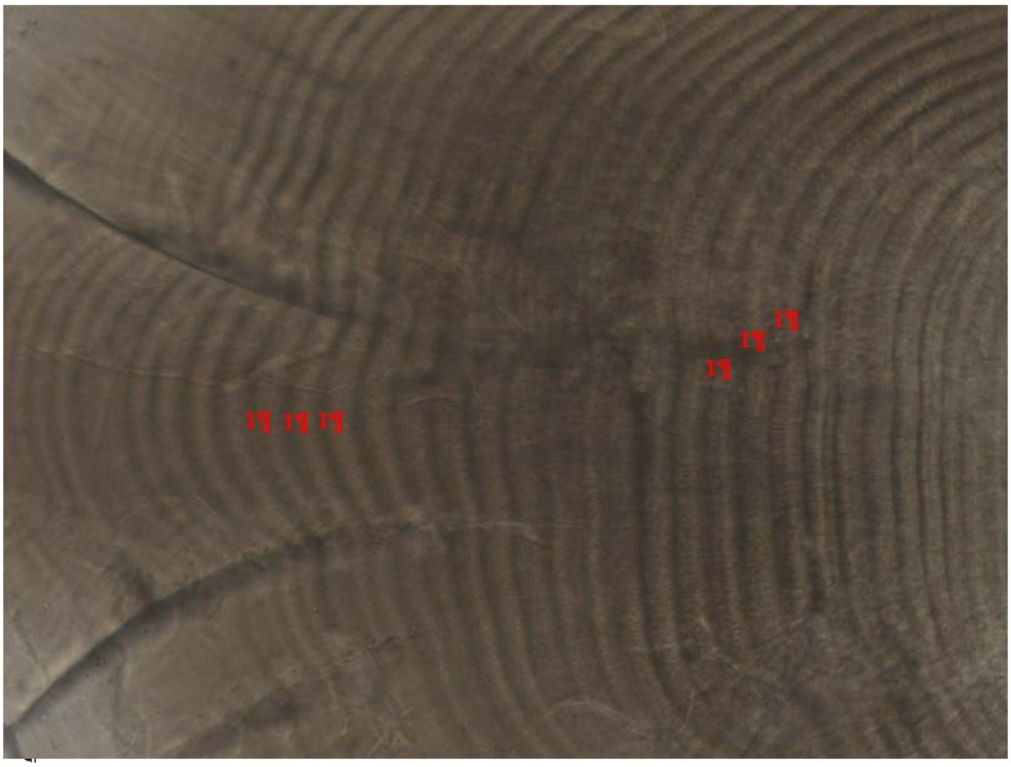


Figure 8: Juvenile otolith from the North Aegean Sea (x200 magnification, Focus 1).

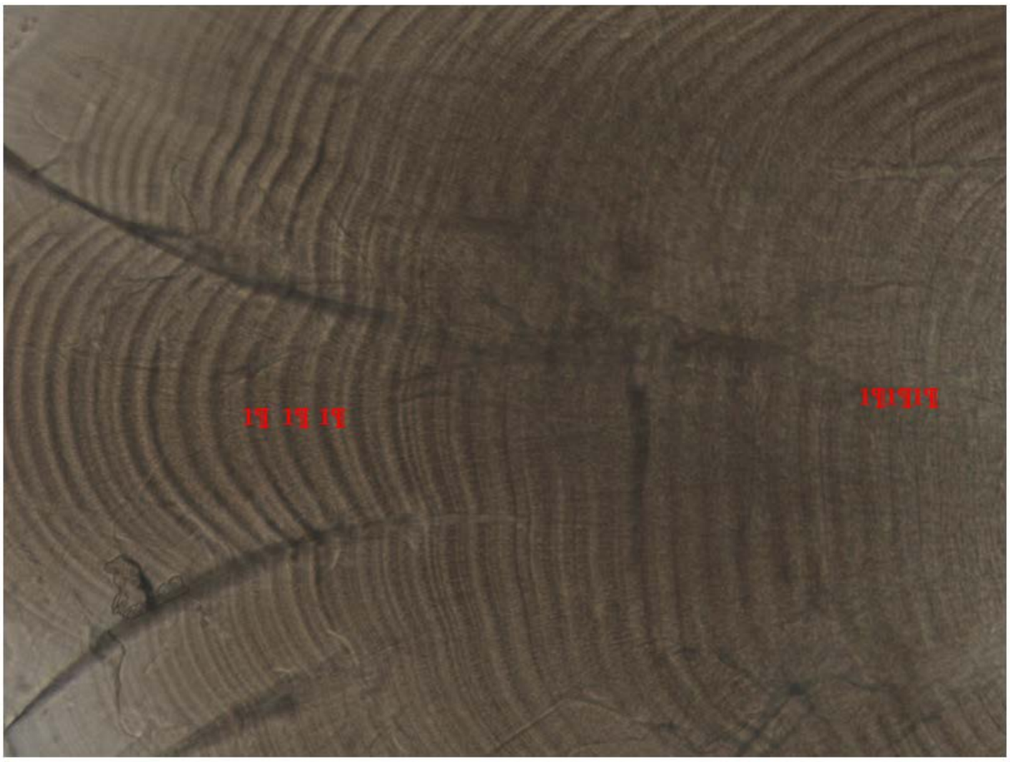


Figure 9: Juvenile otolith from the North Aegean Sea (x200 magnification, Focus 2).

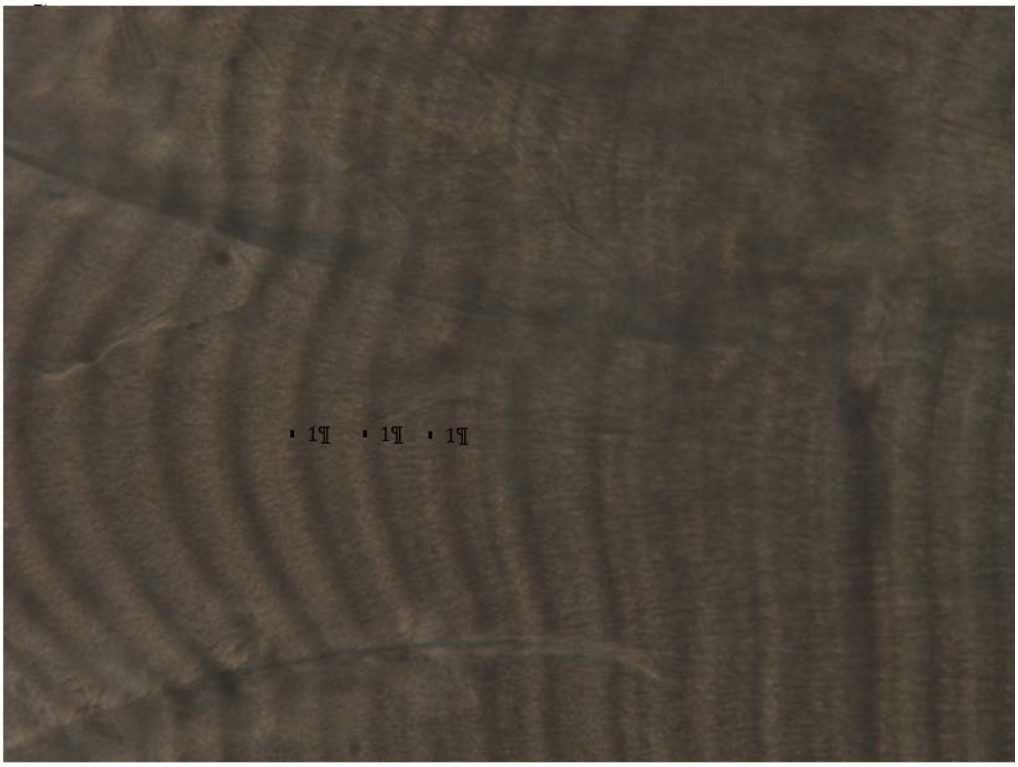


Figure 10: Juvenile otolith from the North Aegean Sea (x400 magnification, Focus 1).

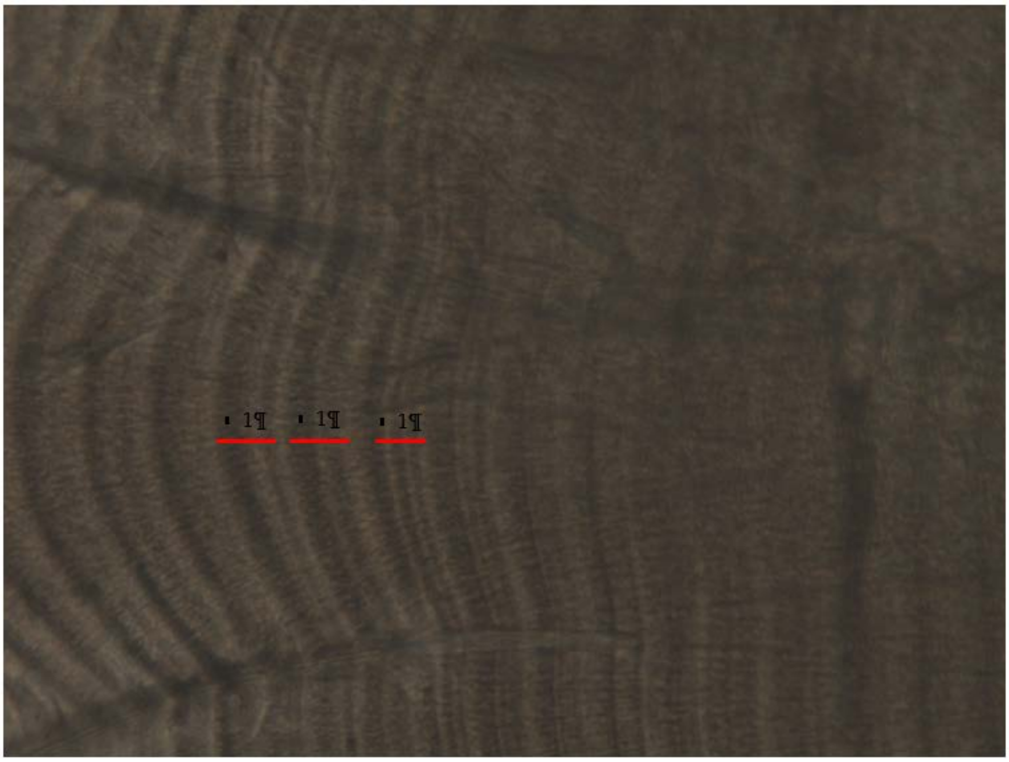


Figure 11: Juvenile otolith from the North Aegean Sea (x400 magnification, Focus 2).

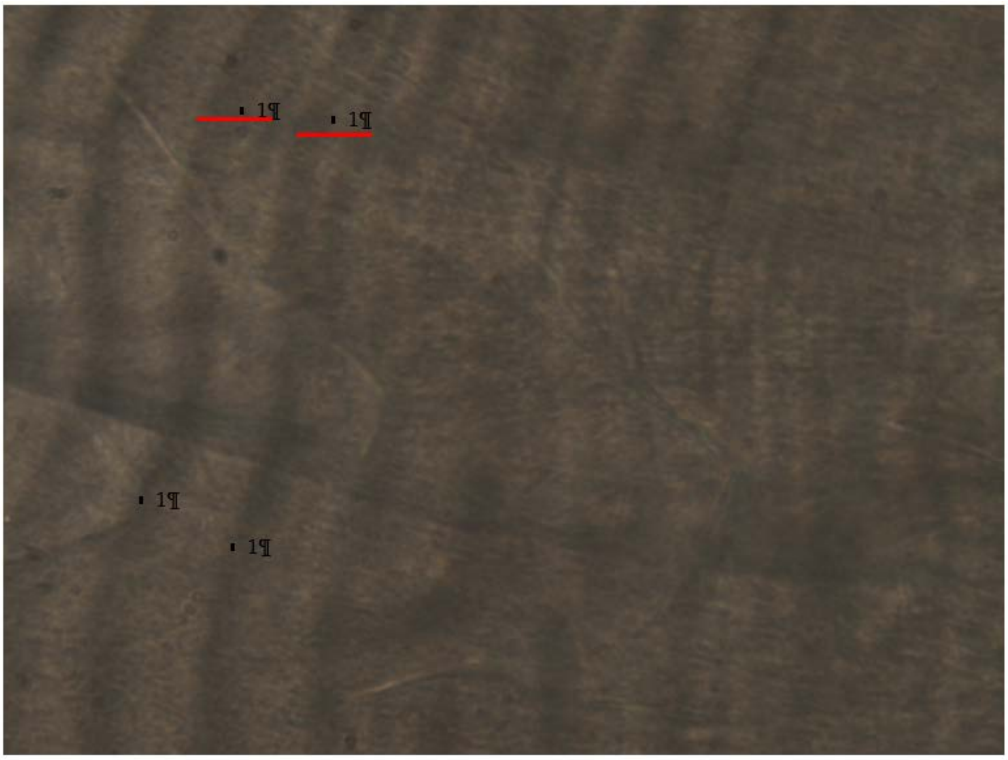


Figure 12: Juvenile otolith from the North Aegean Sea (x630 magnification, Focus 1).

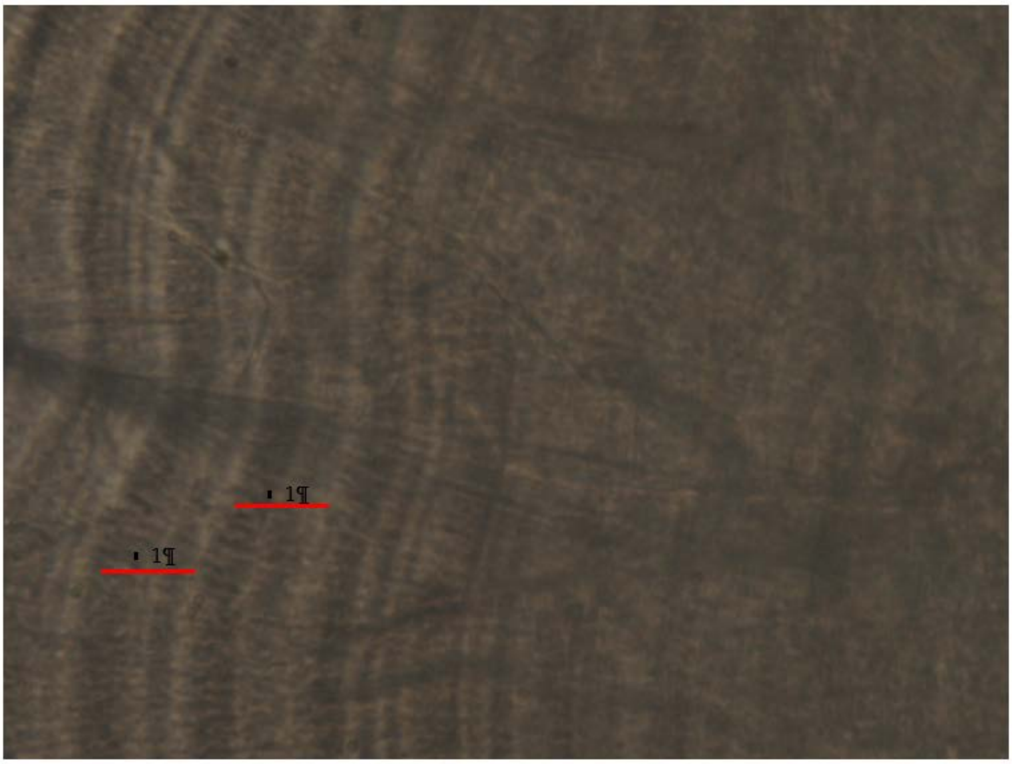


Figure 13: Juvenile otolith from the North Aegean Sea (x630 magnification, Focus 2).

Anchovy juveniles otoliths from the Strait of Sicily

The otolith was read at x200, x400, x630 magnifications. For 67% of the readers the x200 magnification was the best, while a 33% preferred the x400 magnification. Moreover, for this area, readers differed in the reading criteria: 67% of the readers applied the GBR and the 33% applied IMR.

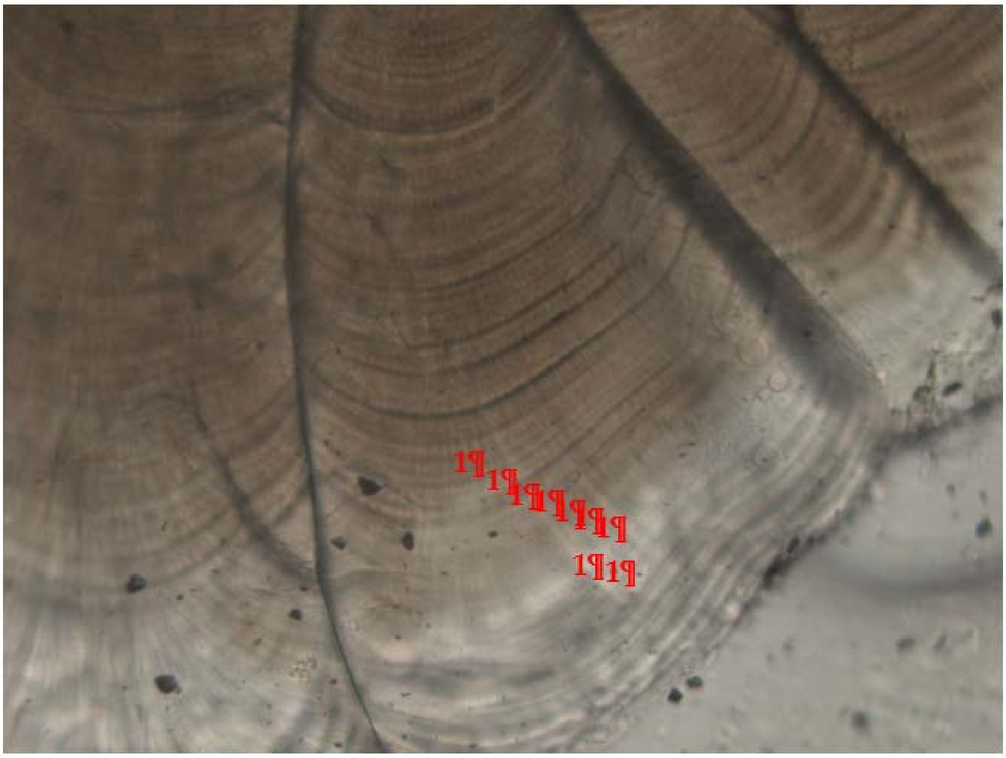


Figure 14: Juvenile otolith from the Strait of Sicily (x200 magnification).Figure 15: Juvenile otolith from the Strait of Sicily (x400 magnification).

Figure 15: Juvenile otolith from the Strait of Sicily (x630 magnification).

Table 2 Anchovy readings agreements

		Ageing Criteria		Magnification			
Stage	Area	GBR (%Ag.)	IMR (% Ag.)	200X (%)	400X (%)	630X (%)	1000X (%)
Larvae	Western Mediterranean	100					100
Juveniles s	Adriatic Sea	67	33	67	33		
Juveniles	Bay of Biscay	100		100			
Juveniles	North Aegean Sea	100		100			
Juveniles	Strait of Sicily	67	33	67	33		

Annex 7: Results of sardine otolith reading exercise during WKMIAS

Workshop on Micro increment daily growth in European anchovy and sardine (WKMIAS). Mazara del Vallo, Italy, 21-25 October 2013

Results of Sardine reading exercise during WKMIAS meeting

During the meeting, an inter-calibration exercise of sardine larvae and juveniles otoliths microstructure analysis was carried out in the laboratory. Ten readers from six institutes with different experience level were involved in the exercise (see Table 1). The exercise was held on 22nd October 2013. The main goal was to agree on a common protocol to be used by all readers in each area. For that reason one otolith was selected from each institute (area) in order to see the possible differences of ageing interpretation. Next reason was the achievement of a potential agreement on magnification used during the reading process, to be able to compare the results by areas in future.

Table 3 – Participants in the sardine exercise

Country/Laboratory	Participants in exchange	Experience level
Spain-Atlantic/ IEO (C. O. Vigo)	Isabel Riveiro	low
Spain-Atlantic/ IEO (C. O. Vigo)	Carmen Piñeiro	low
Portugal – North Portuguese Coast/ IPMA	Andreia V. Silva	low
Spain-Mediterranean/ IEO (Malaga)	Jose Mª Quintanilla	high
Italy-Sicily/ CNR-IAMC	Salvatore Mangano	low
Italy-Ancona/ CNR-ISMAR	Fortunata Donato	high
Italy-Ancona/ CNR-ISMAR	Monica Panfili	high
Italy-Ancona/ CNR-ISMAR	Elisa Domenella	low
Greece/ HCMR	Eudoxia Schismenou	low
Slovenia/FRIS	Tomaz Modic	low

Sardine larvae otoliths from Western Mediterranean Sea

Otoliths were read using x1000 magnification, all readers agreed that this magnification was the best for sardine larvae. Daily increment criteria used by readers during the intercalibration was the Group Band Reading (GBR) (Cermeño *et al.*, 2008). All readers agreed with this criterium.

During the exercise, different focuses were used to read daily rings in different zones of the otolith.

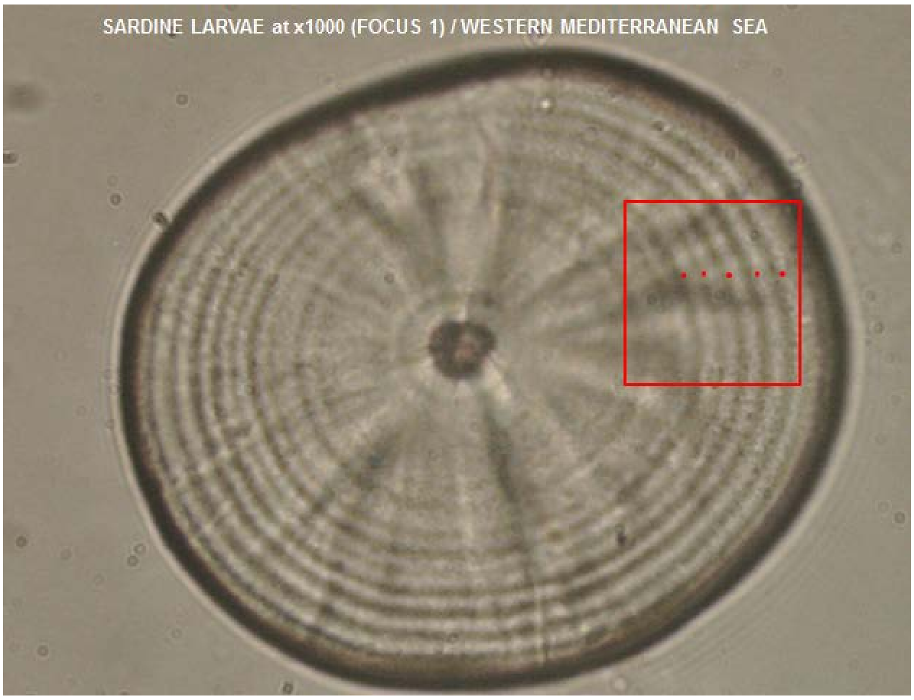


Figure 16 – Sardine larvae at x1000 using focus 1

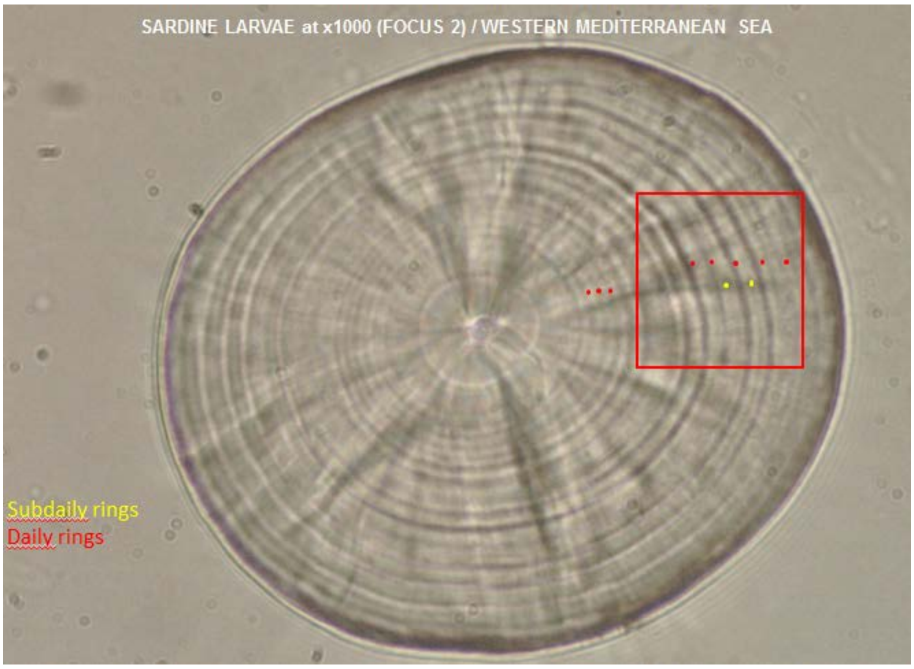


Figure 17 – Sardine larvae at x1000 using focus 2

Sardine juveniles otoliths from Adriatic Sea

Otoliths were read using x200, x400, x630, magnifications. 60% of the readers agreed that x200 was the best magnification for sardine juveniles, and 40% of the readers agreed that x400 was the best. All the readers used both criteria IMR and GBR for daily increments. All readers agreed with the IMR criterium.

Figure 18 – Sardine juvenile at x200 magnification for Adriatic Sea

Figure 19 – Sardine juvenile at x400 magnification for Adriatic Sea

Figure 20 – Sardine juvenile at x630 magnification for Adriatic Sea

Sardine juveniles otoliths from Aquaculture

Otoliths were read using x200, x400, x630, magnifications. All readers agreed that x200 magnification was the best for sardine juveniles because the higher magnifications (x400, x630) did not improve the reading and could lead to an overestimation of daily rings. All the readers used both criteria IMR and GBR for daily increments. Daily increment criteria used by readers during the intercalibration was the Group Band Reading (GBR). All readers agreed with this criterium.

Figure 21-Sardine juvenile at x200 magnification from aquaculture sample in Atlantic Iberian

Figure 22-Sardine juvenile at x400 magnification from aquaculture sample in Atlantic Iberia

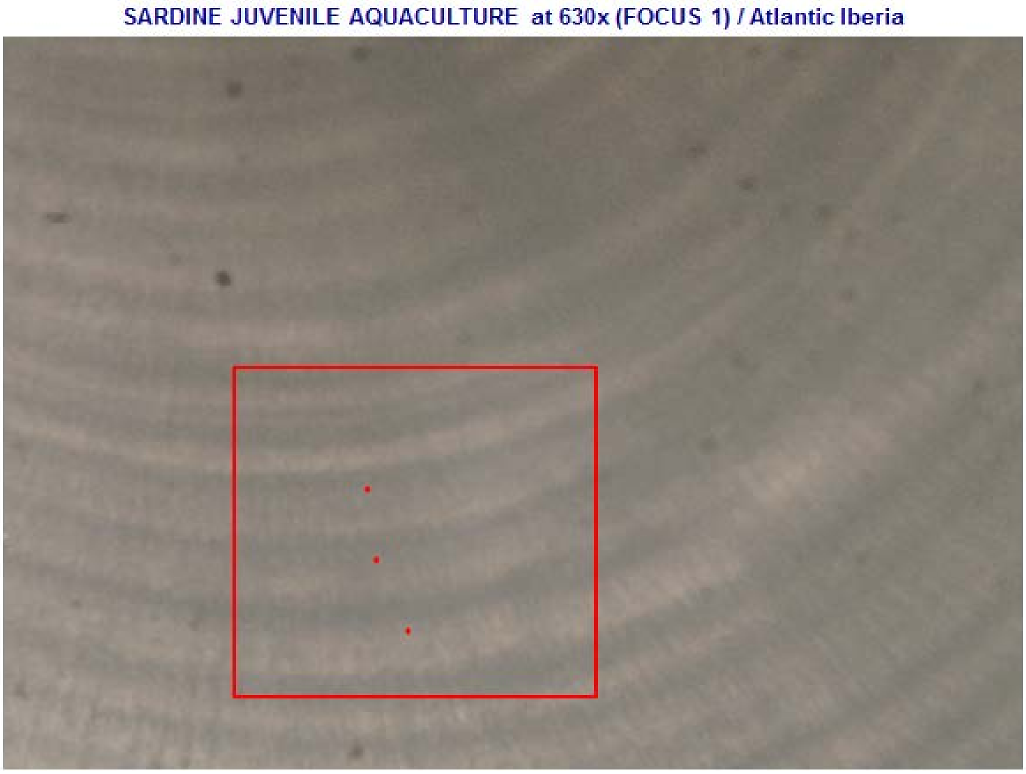


Figure 23- Sardine juvenile at x630 magnification from aquaculture sample in Atlantic Iberia using focus 1

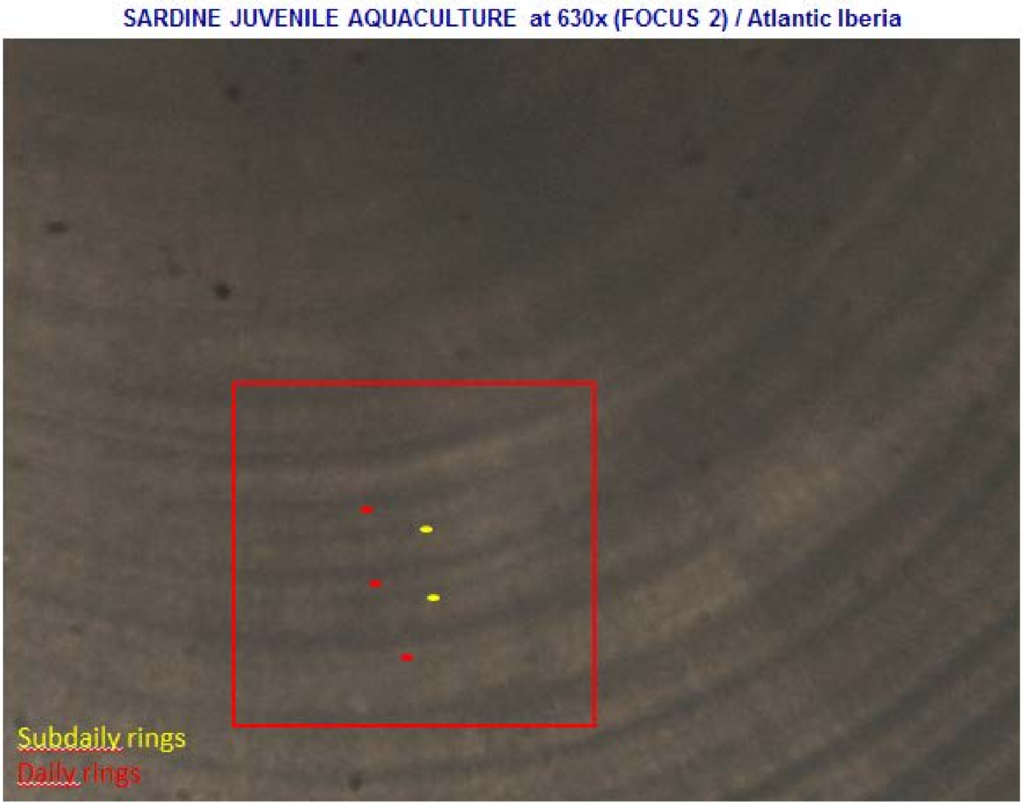


Figure 24- Sardine juvenile at x630 magnification from aquaculture sample in Atlantic Iberia using focus 2.

Sardine juveniles otoliths from North Aegean Sea

Otoliths were read using x200, x400, x630, magnifications. All readers agreed that x200 magnification was the best for sardine juveniles because the higher magnifications (x400, x630) did not improve the reading and could lead to an overestimation of daily rings. Daily increment criteria used by readers during the intercalibration was the Group Band Reading (GBR). All readers agreed with this criterium.

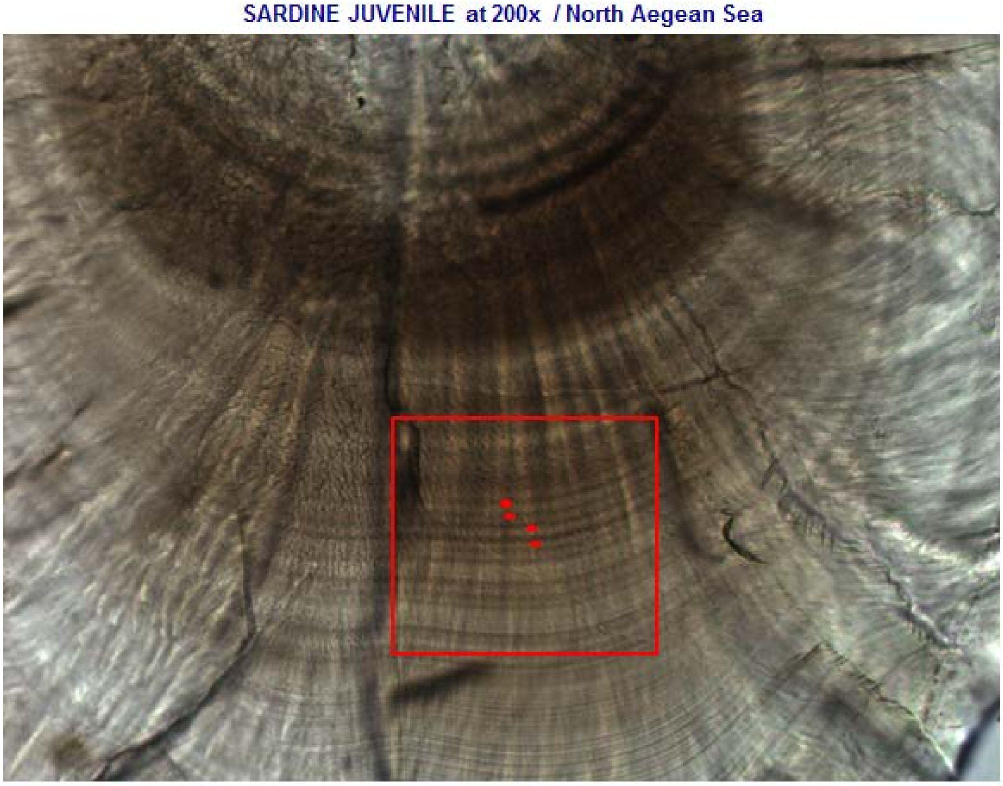


Figure 25 Sardine juvenile at x200 magnification from North Aegean Sea.

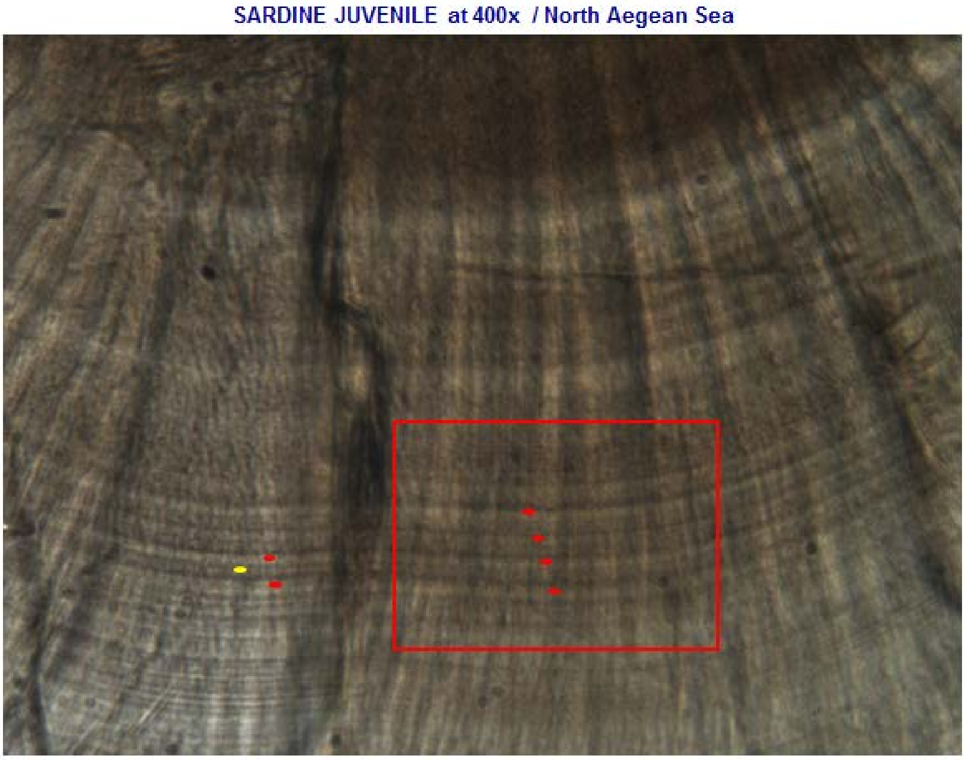


Figure 26-Sardine juvenile at x400 magnification from North Aegean Sea.

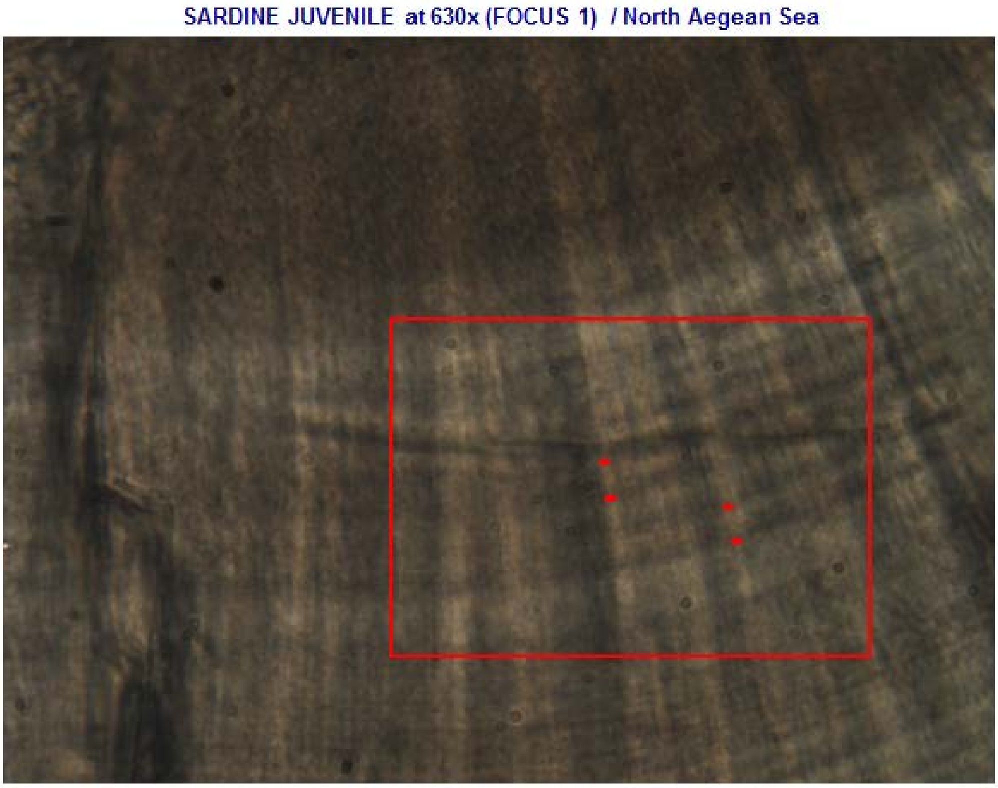


Figure 27-Sardine juvenile at x630 magnification from North Aegean Sea using focus 1.

SARDINE JUVENILE at 630x (FOCUS 2) / North Aegean Sea

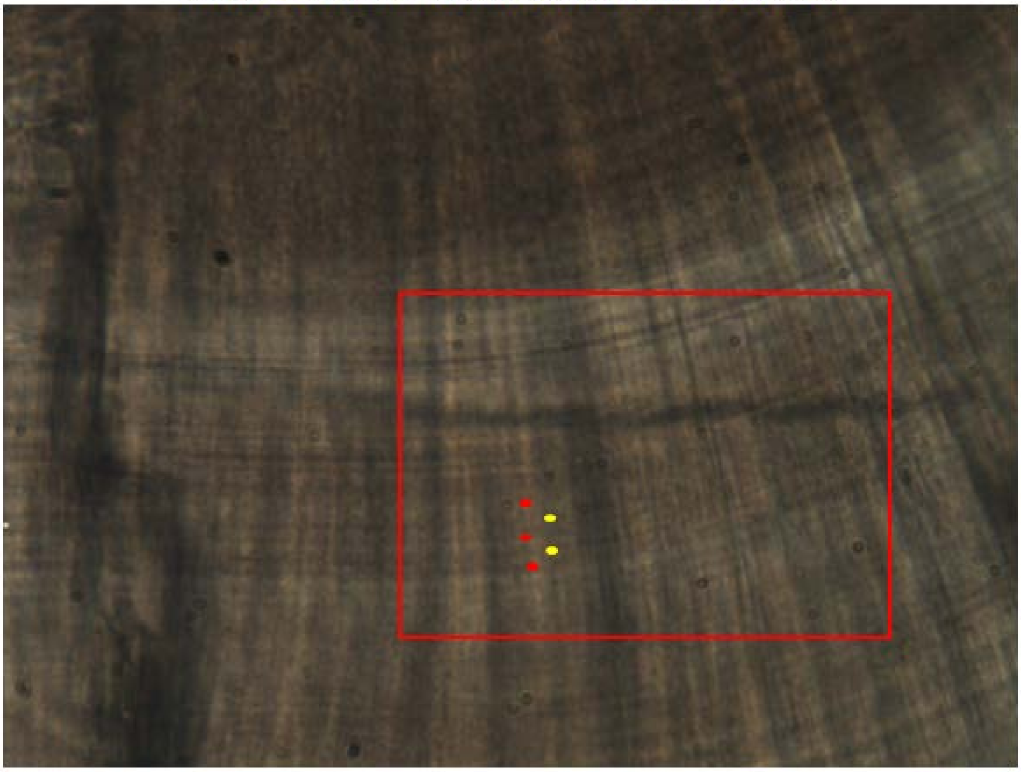


Figure 28-Sardine juvenile at x630 magnification from North Aegean Sea using focus 2.

Sardine juveniles otoliths from North Portuguese Coast

Otoliths were read using x200, x400, x630, magnifications. All readers agreed that x200 magnification was the best for sardine juveniles because the higher magnifications (x400, x630) did not improve the reading and could lead to an overestimation of daily rings. Daily increment criteria used by readers during the inter-calibration was the Group Band Reading (GBR). All readers agreed with this criterium.

For the reduction of the difficulties of the reading the immersion oil was also used for all magnification. The immediate results and the differences are visible in the Figures 29 and 30.

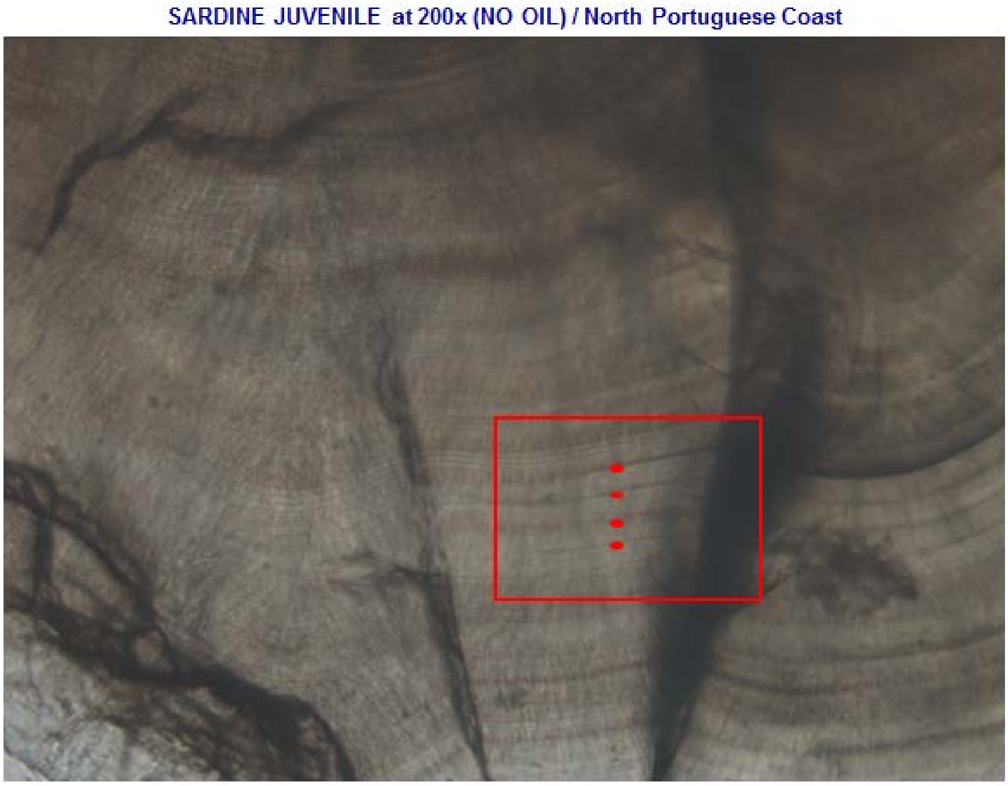


Figure29-Sardine juvenile at x200 magnification from North Portuguese coast.

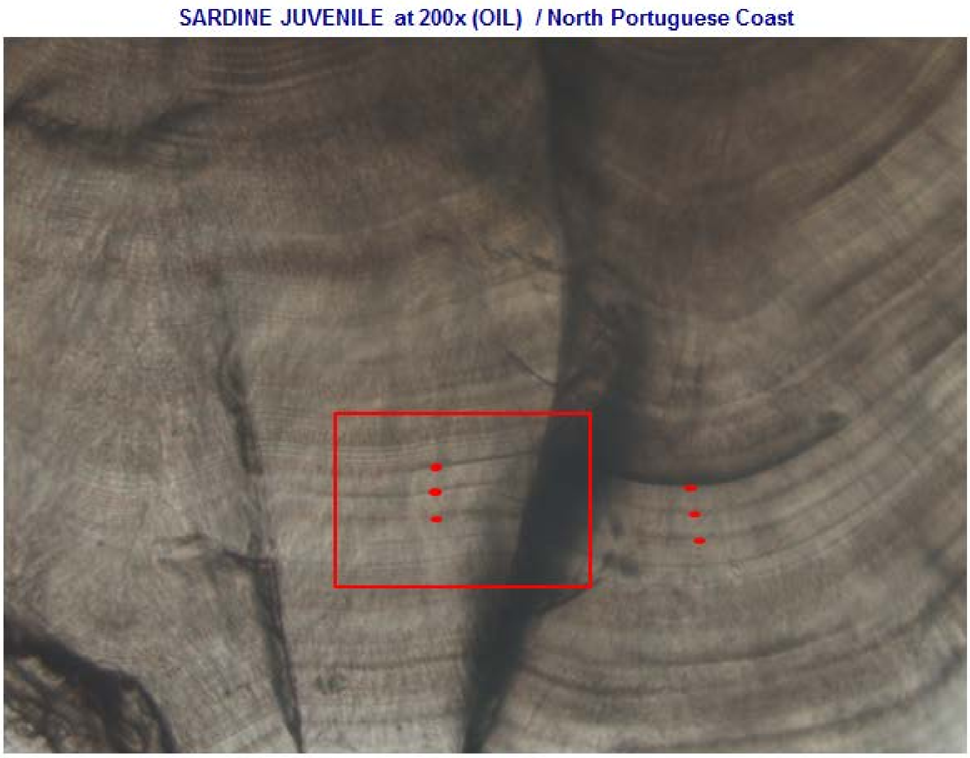


Figure 30- Sardine juvenile at x200 magnification from North Portuguese coast using oil.

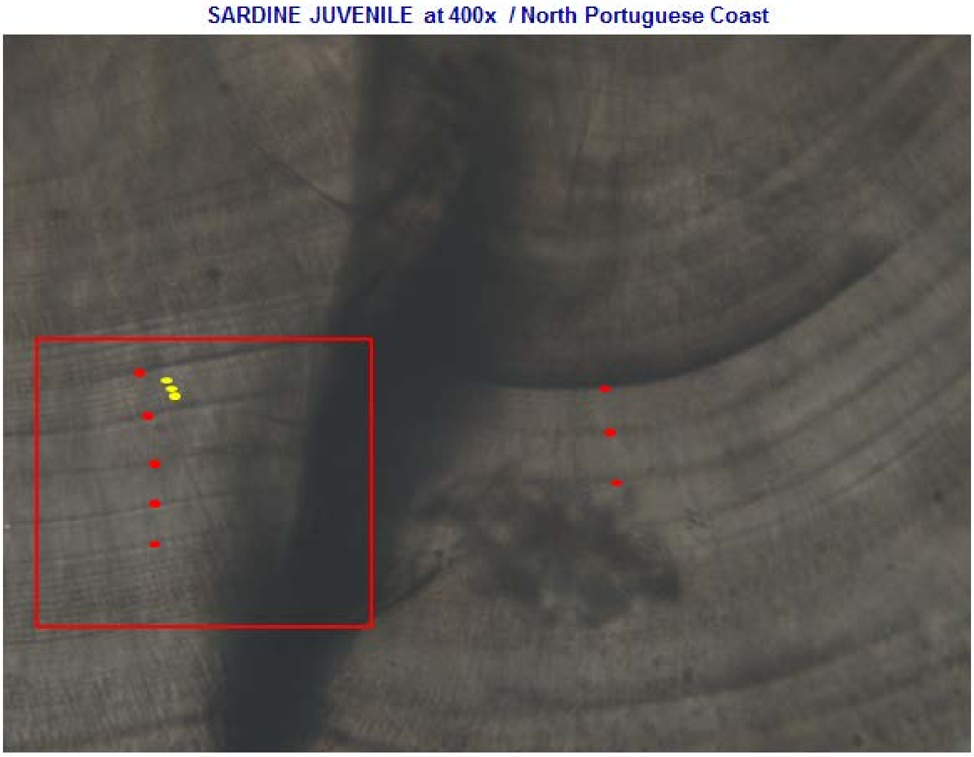


Figure 31-Sardine juvenile at x400 magnification from North Portuguese coast.

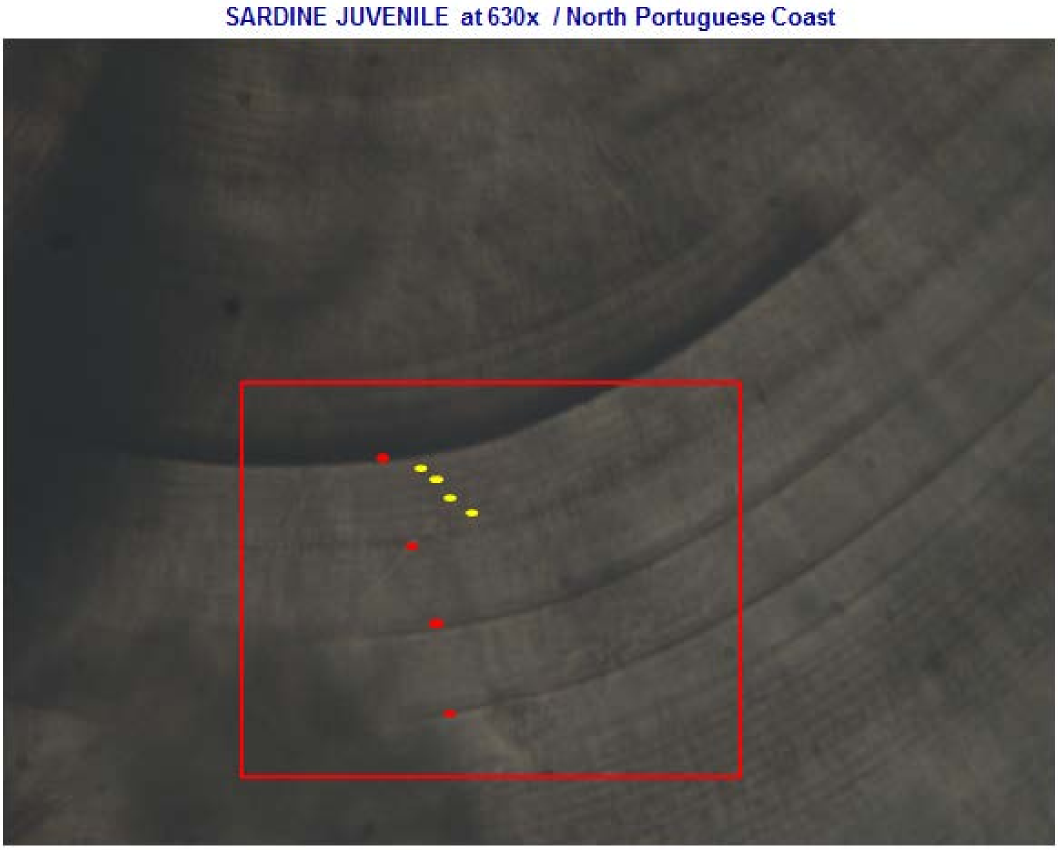


Figure 32-Sardine juvenile at x630 magnification from North Portuguese coast.

Sardine juveniles otoliths from Strait of Sicily

Otoliths were read using x200, x400, x630, magnifications. 90% of the readers agreed that x200 was the best magnification for sardine juveniles, and 10% of the readers agreed that x400 was the best. All the readers used both criteria IMR and GBR for daily increments. All readers agreed on the GBR criterium.

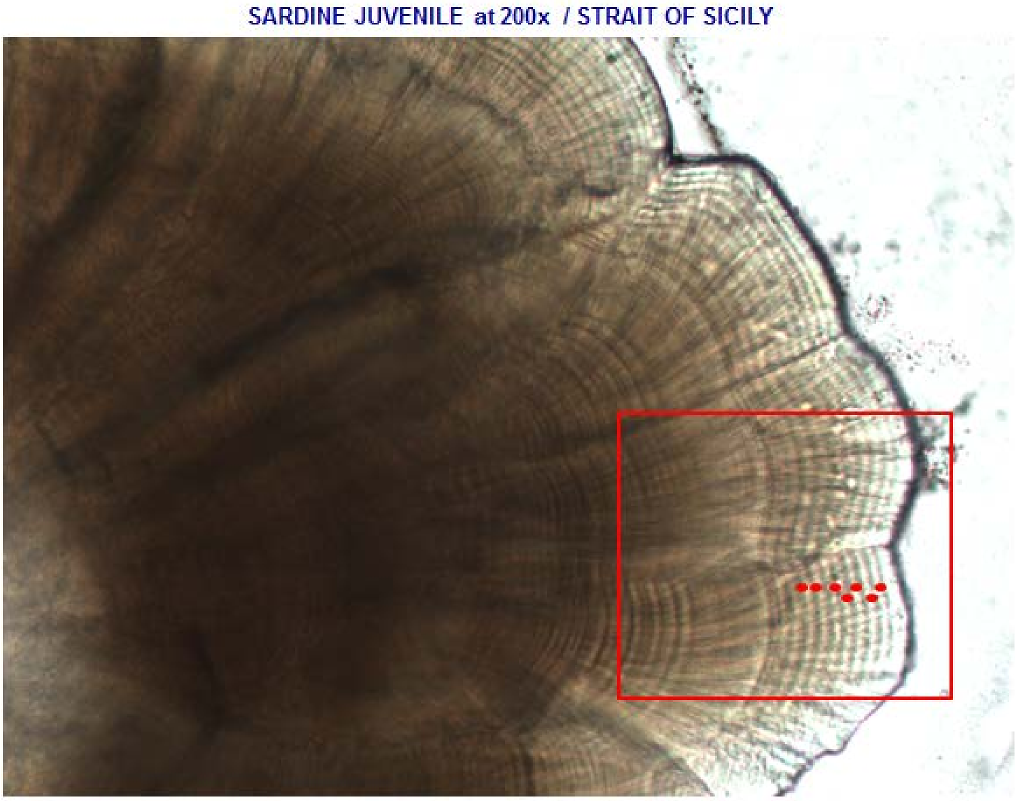


Figure 33-Sardine juvenile at x200 magnification from Strait of Sicily.

SARDINE JUVENILE at 400x / STRAIT OF SICILY

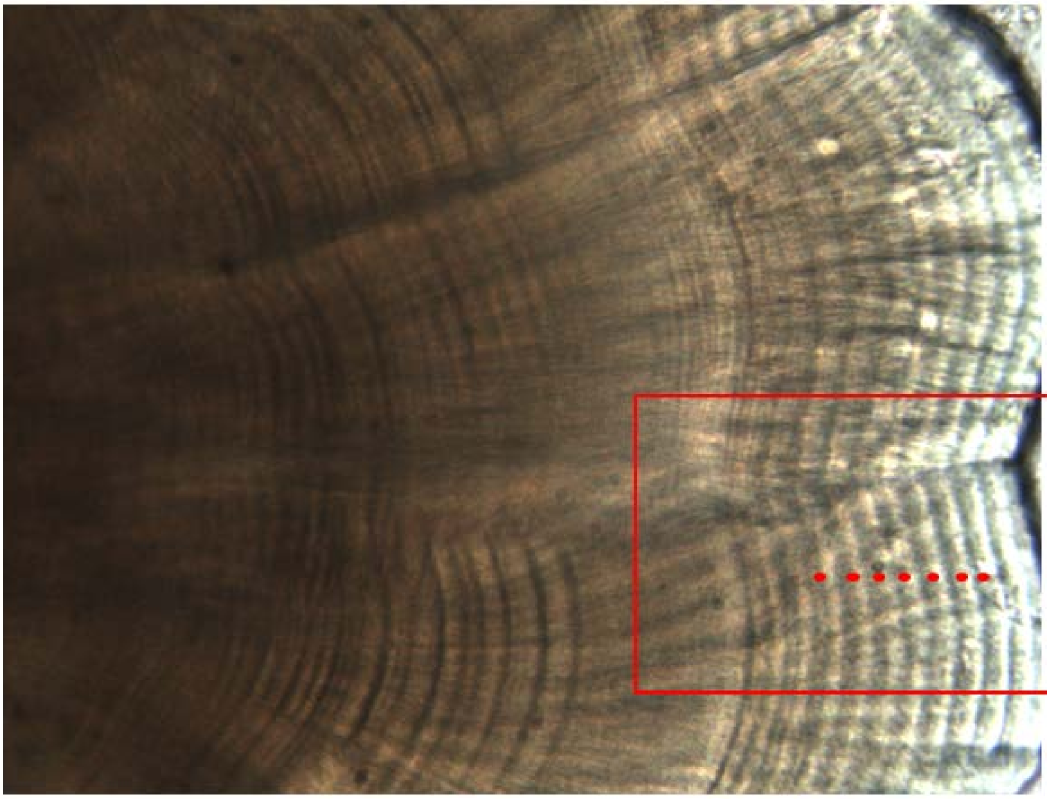


Figure 34-Sardine juvenile at x400 magnification from Strait of Sicily.

SARDINE JUVENILE at 630x / STRAIT OF SICILY

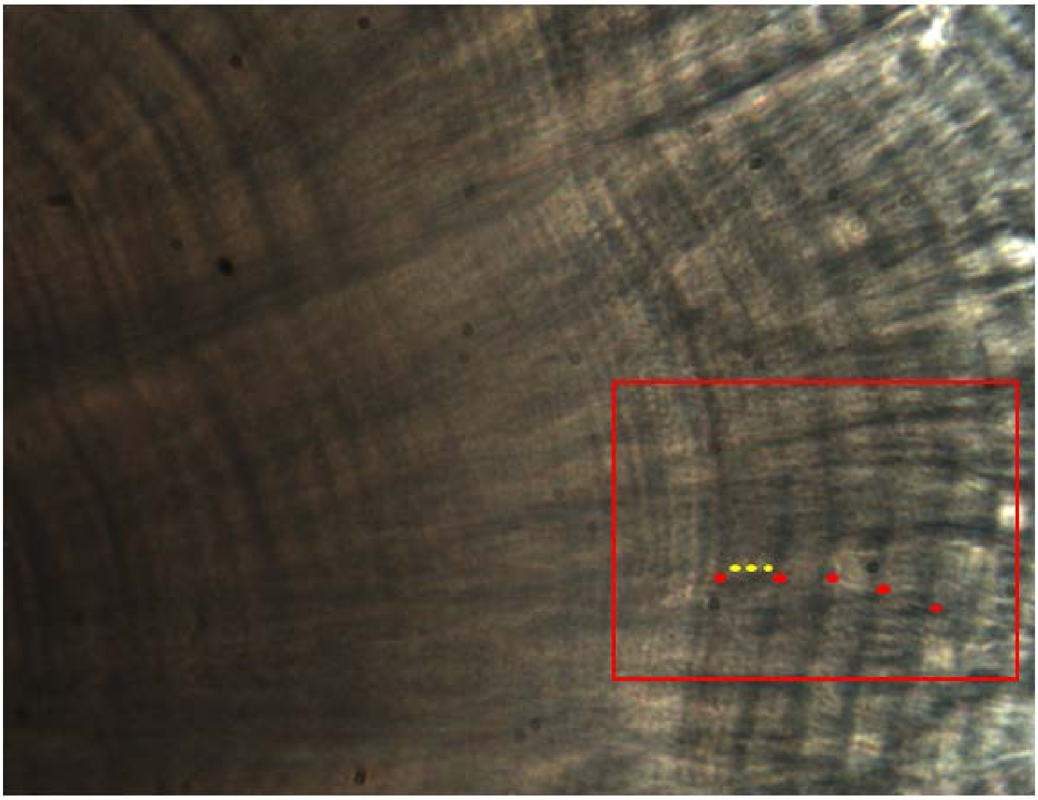


Figure 35-Sardine juvenile at x630 magnification from Strait of Sicily.

These results are summarized in table 4.

Table 4 – Sardine readings agreements

		Ageing Criteria		Magnification			
Stage	Area	GBR (%AG.)	IMR (%AG.)	200X (%)	400X (%)	630X (%)	1000X (%)
Larvae	Western Mediterranean	100					100
Juveniles	Adriatic Sea		100	60	40		
Juveniles	Atlantic-Acquaculture	100		100			
Juveniles	North Aegean Sea	100		100			
Juveniles	North Portuguese coast	100		100			
Juveniles	Strait of Sicily	100		90	10		

Annex 8: Questionnaire sent to the participants before WKMIAS

Questionnaire of WKMIAS 2013

1. Your name (s):
2. Your country:
3. Your institute:

Species (anchovy/sardine) and geographical areas

4. Species used for Micro increment daily growth studies.
5. Does the otolith microstructure come from larvae, postlarvae, juveniles or pre-recruits?
6. Geographical areas.
7. How is the daily age dataset used, e.g. age validation, environmental studies, other (please specify)?

Material and techniques development

8. Pair of otoliths for daily growth studies that you use.
9. How are otoliths stored before treatment?
10. Description of your types of otolith microstructures preparation (e.g. whole otoliths mounted in clear resin on custom plastic slides, sectioned and polished otoliths, etc.).
11. List of your saws/polisher machine (name, manufacturer, description).
12. Description and characteristics of your resin(s).
13. How are otolith microstructure preparations stored?
14. Documents presenting the summary of the techniques used (reference).

Methods in images processing

15. What image capture/analysis software do you use in your laboratory?
16. Which type of camera do you use (digital or analogic, description, characteristics)?
17. Do you calibrate your images?
18. What is the format of images?

Daily age determination

19. Please list the criterion used to estimate daily age (species, geographical area, reference if possible).

Validation methods

20. List of validation of the daily growth ring formation studies (species, geographical area, reference if possible).
21. List of validation of the first annual growth ring studies from the daily increments (species, geographical area, reference if possible).

Information on the quality status of the daily age reading

22. Do you have an internal quality control (description, references)?
23. What age calibration Exchanges or Workshops has your institute participated in during the last three years?