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Report of the Workshop on Age estimation of European anchovy (*Engraulis encrasicolus*)

28 November - 2 December 2016

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Executive summary

Based on the results of a full-scale otolith exchange held in 2014, the Working Group on Biological Parameters (WGBIOP 2015) identified the need for an age reading workshop on European Anchovy otoliths (WKARA2). This workshop (chaired by Andres Uriarte, Spain, Begoña Villamor, Spain and Gualtiero Basilone, Italy), was held in Pasaia, Guipuzcoa (Spain) from the 28 November to 2 December 2016. Five countries took part in this workshop (Spain, Italy, Croatia, Greece and Tunisia), with a total of 16 participants from 9 laboratories. In total 17 areas/stocks were analysed (4 from the Atlantic area and 13 from Mediterranean Sea)

The aim of this workshop was to review the information on age determination, discuss the results of the previous exchange (2014), review the validation methods existing on these species, clarify the interpretation of annual rings and update the age reading protocol and a reference collection of well-defined otoliths.

Age validation studies, in the Bay of Biscay and preliminary validation studies in Division 9a, Alboran Sea and Strait of Sicily areas were presented, including a compilation of age validation studies of this species as well in the literature. There are several areas/stocks in which validations of the anchovy annual age determination have not been done yet.

Due to the poor percentage of agreement achieved in the 2014 Exchange (mean agreement of 66%; mean CV of 58%), the workshop proceeded with a detailed and joint discussion on the growth patterns shown by otoliths from the different areas to find out the major reasons for discrepancies in age determination among readers. At the same time, the joint discussion allowed a better understanding of the pattern of otolith growth increments by areas to improve the guidelines for their interpretation. The discussions on examples among otoliths which generated discrepancies in the age determination led to conclude that there were two major sources of disagreements: a) Divergent otolith interpretation: different interpretations of the marks, growth bands and edges in terms of their conformity with the expected growth pattern of the anchovies, seasonal formation of the otolith by ages and most common checks. and b) wrong application of the age allocation Rules: it was evidenced during the workshop that for the birthdate first July (or first June) in some cases the age determination rule was not being correctly applied during the first half of the year (from January to June).

Following the workshop discussions there has been a progressive change in the perception of the growth pattern applicable to these anchovy otoliths in many areas which led to some revisions of the otolith interpretation and assigned ages, by which growth at ages 0 and 1 are far prominent than at older ages and the occurrence of checks became more frequently admitted. Furthermore, there have been evidences that the age determination rules have in some instances been inconsistently applied. All these evidences led to conclude on the need to review past age determinations. Although this task should be delayed until running an exchange in 2018 to be sure that all the readers apply the protocol and the current criteria of this workshop coherently, since current criteria would change the otoliths interpretation and the age determination in many areas. In addition, for the Mediterranean regions the convenience of midyear birthdates was put in question in comparison with the simplicity of the conventional birthdates at first of January (as these anchovies are in the northern hemisphere).

As a corollary of the former statements, intercalibration exercises by areas, for the different countries taking part in the age reading of the same exploited stock, are still required.

Finally, this Workshop adopted a common protocol for all areas in order to standardize the anchovy age assignments and to improve the coherence of the age estimates. An agreed collection of otoliths by areas were produced and upload to the Age Readers Forum.

1 Introduction

1.1 Terms of reference

A **Workshop on Age estimation of European anchovy (*Engraulis encrasicolus*)** (WKARA2) chaired by Andrés Uriarte, Spain, Begoña Villamor, Spain, and Gualtiero Basilone, Italy, will be established and will meet in San Sebastian (Spain), 28 November–2 December 2016 to:

- a) Review information on anchovy age estimations, otolith exchanges, workshops and validation work done so far;
- b) Analyse growth increment patterns in anchovy otoliths and continue to improve the guidelines for their interpretation;
- c) Analyse the results of the exchanges carried out in 2014 and the potential source of discrepancies, in light of ToRs a) and b);
- d) Increase existing reference collections of agreed aged otoliths.
- e) Address the generic ToRs adopted for workshops on age calibration (see 'WGBIOP Guidelines for Workshops on Age Calibration')

WKARA2 will report by 9 January 2017 for the attention of WGBIOP, SCICOM and ACOM.

A pre-workshop exchange took place in 2014–2015

1.2 Participants

A list of the all participants to this workshop is shown in Annex 2.

A total of 16 readers participated in the present Workshop, 8 from Spain [6 from IEO (laboratories of Santander, Cadiz and Malaga) and 2 from AZTI], 3 from Italy IAMC-CNR, 2 from Greece (HCMR and ELGO), 1 from Croatia (IOF) and 2 from Tunisian (INSTM). Also, one reader from COISPA (Italy) participated by Webex. A list of the participants with a summary about their experience in age estimation of anchovy and the area where they are readers is shown in the Table 1.2.1. The level of experience in anchovy reading was considered by number of otoliths (1st) and by years of experience in this species (2nd). Fourteen of the participants to this workshop are readers for the assessment of anchovy.

Twelve of WKARA2 participants also took part in the last otolith exchange (2014–2015). Seven of them have also participated in the 2009 Exchange and WKARA 2009. The readers of IPMA (Portugal) and ZZRS (Slovenia) participated in the last otolith exchange (2014–2015) but could not assist to the Workshop. Ifremer readers who participated in the exchange of 2014 did not participate in the WKARA2; there are new readers of anchovy in this institute.

In summary eight readers who participated in the exchange have not participated in the workshop. Five new readers, 3 new institutes (IOF, INSTM, and HCMR) and 3 new areas/stocks (Morocco waters, Adriatic Sea, and Tunisian waters) were added to WKARA2.

Table 1.2.1: Summary of WKARA2 participants and reading experience of anchovy otoliths.

Country-Institute	Participants in Workshop 2016	Role	Expertise level/years	Expertise level/years	Participation in Exchange 2014	Participation in Workshop 2009	Participation in Exchange 2009	Area Expertise	Reads for assessment
Spain-IEO Santander	Begoña Villamor	Co-chair	Expert	> 20	Yes (coordinator)	Yes (Co-chair)	Yes (coordinator)	VIII_Bay of Biscay & IXa North	Yes
	Clara Dueñas	Reader /CD	Expert	> 5	Yes	Yes	Yes	VIII_Bay of Biscay & Ixa North	Yes
	Ana Antolínez	Reader /AA	Trainee	1	Yes	No	Yes	VIII_Bay of Biscay & Ixa North	No
Spain-IEO Cadiz	Verónica Duque	Reader / VD	Trainee	1	No	No	No	Morocco: North Atlantic area	Yes
	Jorge Tornero	Reader /JT	Expert	> 5	Yes	Yes	Yes	IXa S_Gulf of Cadiz	Yes
Spain-IEO Malaga	Pedro Torres	Reader /PT	Expert	> 10	Yes	Yes	Yes	GSA01_Alboran Sea & GSA06_Western Mediterranean	Yes
Spain-AZTI	Andrés Uriarte	Co-chair/ Reader /AU	Expert	> 20	Yes (coordinator)	No	No	VIII_Bay of Biscay	yes
	Iñaki Rico	Reader /IR	Expert	> 20	Yes	Yes	Yes	VIII_Bay of Biscay	Yes
Italy-IAMC-CNR	Gualtiero Basilone	Co-chair	Expert	>20	Yes	Yes (Co-chair)	Yes (coordinator)	GSA16: Strait of Sicily	Yes
	Salvatore Mangano	Reader /SM	Intermediate	<5	Yes	No	No	GSA09: Tyrrhenian Sea	Yes
	Maurizio Pulizzi	Reader /MP	Intermediate	<5	Yes	No	No	GSA10: Tyrrhenian Sea	Yes
Italy- COISPA	Pierluigi Carbonara (Part of time, via Webex)	Reader /PC	Expert	>10	Yes	No	No	GSA10_Southern Thyrrenian & GSA19_Western Ionian	Yes
Greece - HCMR	Ioannis Fytidakos	Reader /IF	Intermediate	< 5 (4 years)	No	No	No	GSA 22, 20 (Aegean Sea)	Yes
Greece - ELGO	Cristina Milani	Reader /CM	Trainee	2	Yes	No	No	GSA 22 (Aegean Sea) 20 (Ionian Sea)	Yes
Tunisia-INSTM	Adel gaamour	Reader /AG	Intermediate	<5	No	No	No	GSA 12, 13, 14 (Tunisian waters)	Yes
	Sana Khemiri	Reader /SK	Intermediate	<5	No	No	No	Tunisian waters	Yes
Croatia-IOF	Denis Gašparevic	Reader /DG	Intermediate	< 5 (4 years)	No	No	No	GSA 17_North Adriatic Sea	Yes

1.3 Work carried during the workshop

The work carried out during the meeting is detailed in the actual agenda described in Annex 1.

The workshop focused on the TORs a, b, and c.

A serie of presentations with Reviews of available information on anchovy age validations, otolith growth patterns and age estimation procedures for the different areas of study was presented during the first day of the meeting (ToR a and b).

An analysis of the results of the exchange carried out in 2014 was made followed by a detailed presentation of typical otoliths by regions and the several interpretations and age determinations annotated during the 2014 Exchange exercise. This served to illustrate the sources of discrepancies (ToR c). Such an exercise took longer than foreseen (1.25 day) and corroborated that there were major discrepancies in the interpretation of otoliths and in the understanding of the growth pattern of anchovy otoliths between areas and readers. For this reason it was felt that before solving the current discrepancies there was no need to run a new exercise of age determination among the attendees to evaluate any progress during the workshop.

The workshop proceeded afterwards with a more detailed and joint discussion on the growth patterns shown by otoliths from the different areas (for a 1.25 days). While doing this a set of typical (some easy and some difficult) otoliths by areas were analysed jointly on a common screen (via slide projector) and on every particular PC screens. Readers had been asked in advance to bring with them images of otoliths of typical (and easy to read) and others of some difficulty. This common examination and discussion of otoliths by areas allowed finding out the major the reasons for discrepancies in age determination among readers (TOR c). At the same time allowed a better understanding the pattern of otolith growth increments by areas (TOR b) to improve the guidelines for their interpretation. In summary every reader was allowed to make a presentation of the otoliths of their respective areas while allowing direct common discussion on the interpretations. Such an exercise served to propose a set of otoliths for the collections of agreed aged otoliths (TOR d).

The last day of the meeting was devoted to update the age reading criteria based on the validation studies results and agreements after the discussions we had during the workshop, and to go through the general discussions (on manual for age determination of anchovy collection of agreed aged otoliths etc.), structure of the reporting and future research and recommendations, etc.

The group was satisfied with the discussions carried out on their respective otoliths by regions and there was a general feeling that progress had been achieved towards a common understanding of the growth pattern of anchovy otoliths across all regions as reflected in the report.

The potential for a final evaluation of progress in the agreements in age determination was left for a later step.

2 Life history of European Anchovy and stocks/areas: Review and update the information presented in WKARA1

2.1 Life history of European Anchovy

2.1.1 Bay of Biscay

The Bay of Biscay anchovy (*Engraulis encrasicolus*) is the short-lived pelagic fish with the greatest importance to fishing in Spain and France. It is considered as a stock isolated from other small anchovy populations located north and south of the Northeast Atlantic (Magoulas *et al.*, 2006; Zarraonaindia *et al.*, 2012).

Bay of Biscay anchovy is characterized by its seasonal migrations, the intense growth it undergoes throughout its short life (3 or 4 years at most), its early sexual maturity (from its first year of life), its formation in shoals (above all, in the spawning season in spring), and the space it occupies depending on its abundance (the greater the abundance, the larger the spawning ground). One of its important characteristics is the enormous interannual fluctuations in the abundance of its population, with high and variable natural mortality. These fluctuations are due to the great variations in recruitment, driven mainly by environmental factors. Figure 2.1.1.1 shows schematically the life cycle of anchovy (Uriarte *et al.*, 2014).

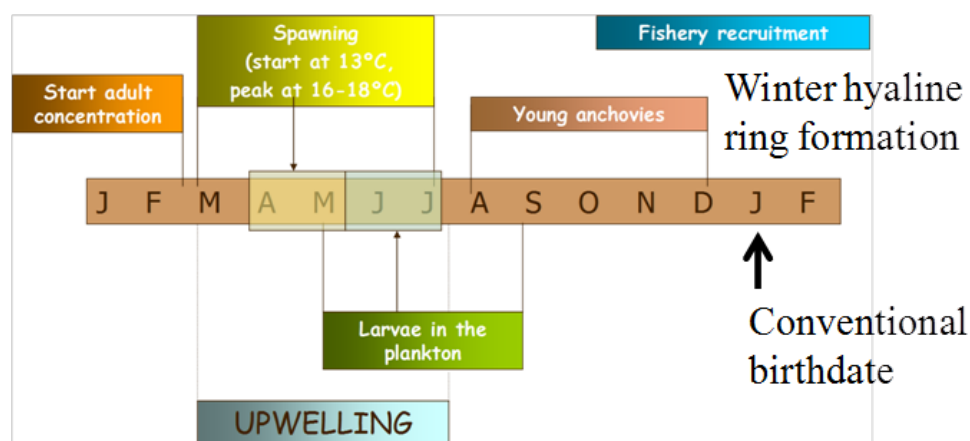


Figure 3.1.1.1. Scheme of the life cycle of the Bay of Biscay anchovy.

As spring and summer progress, anchovies migrate from the interior of the Bay of Biscay towards the north along the French coast and towards the east along the Cantabrian Sea, where they spend the autumn. In winter, they migrate in the opposite direction towards the east and southeast of the Bay of Biscay (Prouzet *et al.*, 1994).

Pelagic habitats within the Bay of Biscay are dynamic and characterized by the presence of a variety of mesoscale physical features such as river plumes, upwelling areas, gyres, eddies, and fronts, with the strength of these features depending upon the season and climatic conditions (Koutsikopoulos and Le Cann, 1996) (See a summary in WKARA 2009 (ICES, 2009)). The anchovy life cycle in the Bay of Biscay is associated with seasonal changes in a variety of the physical features and to specific biological factors (Petitgas *et al.*, 2013).

Many authors have suggested that anchovies reach their first sexual maturity at 1 year old (Furnestin, 1945; Cort *et al.*, 1976; Lucio and Uriarte, 1990; Uriarte *et al.*, 1996), with a size of 11.0 cm for males and 11.5 cm for females.

The anchovy is an indeterminate multiple batch spawners, that is to say that it produces several spawns (lays eggs several times) during the spawning season that extends from April to August (Motos *et al.*, 1996). The moment of greatest spawning intensity occurs between the months of May and June. Larger and/or older anchovies begin to reproduce at the end of April and throughout May (13–14°C) and in more oceanic waters (often associated with the continental slope); the young and/or smaller anchovies spawn later at the end of May and in June (16°C), which normally spawn in shallower waters (Motos *et al.*, 1996). The spawning area is located south of 47°N and east of 5°W. Most of the spawning takes place on the continental shelf, in areas of influence of the rivers plumes whose mouths go to the interior of the Bay of Biscay: Garona, Adour and rivers of the Cantabrian coast (Motos *et al.*, 1996).

The anchovy eggs and larvae of anchovy developed between April and August. Mesoscale processes in relation to the vertical structure of the water column (stratification, upwelling and river plume extent) appear to have a great influence on the survival of larvae (Allain *et al.*, 2001). The larvae are found during summer within stratified waters (Allain *et al.*, 2007; Irigoien *et al.*, 2008) and growth is potentially most rapid above 16°C (Urtizberea *et al.*, 2008). Early juveniles are found in late summer and autumn in stratified waters that are >18°C. Depending on the direction and intensity of currents as well as dispersion, larvae can be transported into many different areas, from nearshore coastal habitats to off-shelf oceanic areas (e.g. Petitgas *et al.*, 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 (Aldanondo *et al.*, 2011). 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. (Uriarte *et al.*, 1996; 2016).

In a study recently, a new parameters and curve of growth equation of von Bertalanffy was estimated and the age determination has been validated for the Bay of Biscay (Uriarte *et al.*, 2016). In this study, the mean lengths-at-age have remained almost constant throughout the time-series (since late 1980s) in the catches of the spring Spanish fishery, with von Bertalanffy growth parameters similar to those calculated by Hernández *et al.* (2009), also for the Spanish fishery, and Vaz *et al.* (2002) for the acoustic surveys in the Bay of Biscay. However, these results are in contrast to those published previously by Cendrero *et al.* (1981) for the Spanish fishery and by Guerauld and Avrilla (1974) in the French area which reported length-at-age 1 of 10 cm and length-at-age 2 of 14.5 cm in catches (i.e. the latter corresponds to values obtained using the method described herein for mean length-at-age 1).

2.1.2 Division 9a

The distribution of anchovy in the Division 9a is nowadays mainly concentrated in the Spanish waters of the Gulf of Cádiz (Subdivision 9a-South). Outside the main nucleus of the Gulf of Cádiz, resilient anchovy populations have been detected in all fishery-independent surveys (ICES, 2007) and previous records on large catches in ICES Areas 9a North, Central North and South (Algarve) suggest that abundance in those areas have been high in early years of the time-series. The anchovy population in Subdivision 9a South appears to be well established and relatively independent of populations in other parts of the Division. These other populations seem to be abundant only when suitable environmental conditions occur. (ICES WGHANSA, 2016. Stock Annex). Therefore, only

the life history of the anchovy in the Gulf of Cadiz will be presented since the information from the other areas is very scarce.

2.1.2.1 Gulf of Cadiz

The Gulf of Cádiz anchovy is mainly concentrated in the Spanish waters of the Gulf of Cadiz (Subdivision 9a-South). The anchovy population in Subdivision 9a-South appears to be well established and relatively independent of populations in others parts of the Division (ICES, 2009a).

The spawning season extends from late winter to early autumn with a peak spawning time occurring from June to August. According to differences in the energy investment in somatic growth and reproductive, larger anchovies are spring-summer spawners whereas anchovies in their first reproduction are summer spawners.

Length at sexual maturity was estimated at about 11 cm. However, it was corroborated that size at maturity may vary between years, suggesting a high plasticity in the reproductive process in response to environmental changes (Millán, 1999). A clear decreasing trend in L_{50} estimates has been observed in the last years, spanning from values of around 11 cm at the beginning of the 1990s to estimates close to 9 cm in the last years.

The estuary of Guadalquivir River is largely used by post-larvae and juveniles of anchovy as a nursery area (Drake *et al.*, 2002; Baldo and Drake, 2002). Anchovy eggs and larvae were mainly detected from March to November showing different spatial patterns. So, while eggs were distributed offshore in front of the Tinto-Odiel River mouth in spring and in summer and in a shallow area between the Tinto-Odiel and the Guadalquivir River mouths, larvae were mainly distributed at the vicinity of the Guadalquivir River mouth (Baldo *et al.*, 2006). Therefore, the eastern sector, under Guadalquivir River influence, can be regarded as highly suitable for anchovy larval development and survival. The growth parameters for anchovy in the Gulf of Cadiz were estimated by using length frequency analysis methods. The mean parameter estimates from 1989 to 1993 period were $L_{\infty} = 18.95$ cm and $k = 0.90$. The high value of the parameter k shows the high growth rate. The Gulf of Cadiz anchovy reaches 60% of its asymptotic length in its first year of life and 83% in its second year of life (Bellido *et al.*, 2000).

With regard to the oceanographical conditions, surface currents in the Gulf of Cadiz are governed by the seasonality of atmospheric forcing and the water exchange at the strait of Gibraltar. The inner shelf dynamics is governed by the balance between alongshore pressure gradients and the local windstress (Relvas and Barton, 2002). The former, dominated by buoyant inputs from the estuarine systems, tend to drive inshore poleward currents although local windstress can either enhance or revert the circulation. The onset of sharp current reversals is therefore frequent (Sánchez *et al.*, 2006) and the average circulation depicts a pair of coherent cyclonic cells separated by Cape St. Maria. The outer shelf is dominated by the so-called Gulf of Cadiz Current (Peliz *et al.*, 2009). This baroclinic, equatorward current partially compensates for the outflow of med waters and is a direct consequence of the water exchange at Gibraltar. This flow occurs year-round and brings Atlantic waters from the Portuguese shelf onto the eastern GoC (Bellanco and Sánchez, 2016). However, it exhibits some seasonal variability with increased breadth and lowest annual temperature and salinity values after the summertime, probably aided by local positive wind curl and upwelling-favourable equatorward winds off Portugal (Bellanco and Sánchez, 2016). Offshore, the mesoscale field is prolific (Peliz *et al.*, 2014) but the mean pattern is characterized by an equatorward current bundle transporting

warmer, more saline, Atlantic waters with properties resembling the Azores Current water (Sánchez and Relvas, 2003). This flow is rather barotropic and feeds the Atlantic inflow through the central and southern Strait of Gibraltar (Peliz *et al.*, 2009).

2.1.3 Alboran Sea

Ecosystem considerations for anchovy populations.

Alboran Sea is located between the Atlantic Ocean and the Mediterranean Sea. There is an exchange of salt and water through the Strait of Gibraltar (Lacombe and Richez 1982; Bryden *et al.*, 1994). This exchange is produced through an incoming Atlantic surface current, which flows eastward, while underneath the Mediterranean waters (more saline and colder) run out into the Atlantic Ocean. Between these two water currents there is an interface that is relatively variable in height. The Atlantic waters are characterized by their low nutrient content, compared to Mediterranean waters that flow through the bottom of the basin (Coste *et al.*, 1988; Minas *et al.*, 1991; Gomez *et al.*, 2000).

From the hydrographical point of view, the Alboran Sea basin is characterized by high dynamism. The Atlantic surface current leads to the formation of mesoscale structures that show high spatial and temporal variability and exert a very direct influence on the continental shelf, particularly of the Northern Alboran Sea coast. This coast also is under the influence of the system of prevailing winds, western and eastern winds (Arevalo and Garcia Lafuente, 1983; Cano and Garcia Lafuente, 1991). The general circulation model shows its influence on the biology of the species and the geo-chemical structures of the water (Rodriguez *et al.*, 1998; Garcia Lafuente *et al.*, 1998).

One of the processes of fertilization of the Alboran Sea is associated with the general pattern of movement of the incoming Atlantic waters. This current generates two almost permanent anticyclonic water gyro in the western basin occupying almost the entire basin (Figure 2.1.3.1). As a result, it creates an intense geostrophic front which is positioned in the margin of the gyro (Minas *et al.*, 1991; Sarhan *et al.*, 2000). This front creates a zone of divergence between the northern margin of the gyro and the continental shelf, creating an upwelling of saline waters and rich in nutrients, visible by the temperature and salinity images captured by satellites. This circulation pattern is modified by the incoming current variability. This variability has a seasonal component (Vargas-Yañez *et al.*, 2002), with maximum occurring in summer and minimum during winter (García Lafuente *et al.*, 2002a).

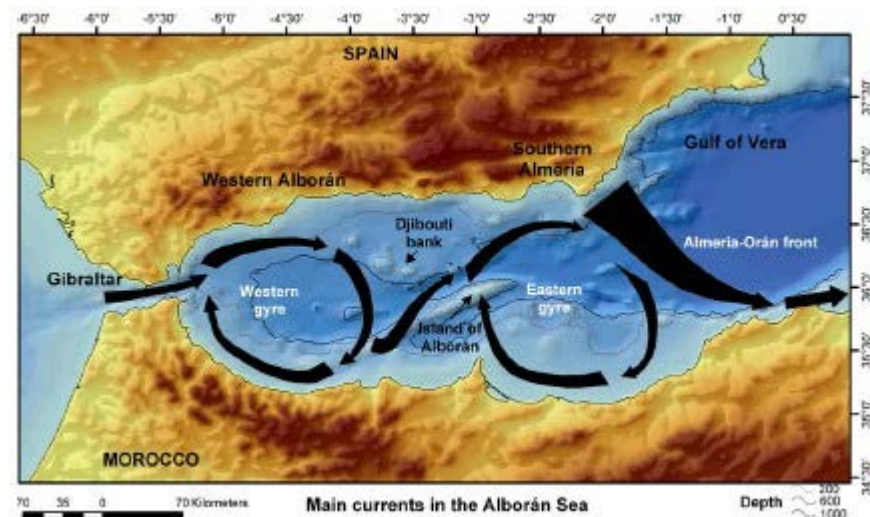


Figure 2.1.3.1. Schematic representation of the gyro in the Alboran Sea basin www.alnitak.info/spanish/alboran/fisica.php

Another water fertilization process is associated with western wind regime along all the coast of the Northern Alboran Sea that produces upwellings periodically (Cano and García Lafuente, 1991; Sarhan *et al.*, 2000). These processes are critical for the larvae of small pelagic, since larvae banks are distributed in shallow coastal areas that are easily influenced by the wind action. The bays of the Northern Alboran Sea, as are the bays of Malaga and Almeria, represent areas of accumulation and retention of larvae, which given their characteristics, are optimal for considering it an essential habitat for small pelagic species (Warner *et al.*, 2000). The influence of wind patterns on larval and juvenile growth in these coastal areas has been corroborated recently (Alemany *et al.*, 2006).

Anchovy fishery in Northern Alboran Sea (GSA01)

Anchovy is the main target species of the purse-seine fleet in Northern Alboran Sea, due to its high economic value, although its abundance is low and very local. Catches in the period 1990–2014 has been highly variable, with a minimum of 157 tonnes in 1993. Higher catches occurred in 1996 and 2001–2002 and 2013 they were caught between 2000 and 3200 tonnes. The whole period average is 888 tonnes. In the early twenties of the last century, anchovy was fished all around the Alboran Sea, but currently Málaga Bay is the only area where anchovy is fished throughout all the year and where more than 80% of catches are located. The fishery of anchovy in the Malaga Bay is exclusively focused on individuals from early age classes because older age classes are not found: almost all the catch corresponds to class 0 and 1. Years with higher catches are usually correlated with a successful and high recruitment period, while unsuccessful recruitment in a given year is correlated with a low level of catch.

Species with a lower economical value are also fished, sometimes representing a high percentage of landings: horse mackerel (*Trachurus* spp.), mackerel (*Scomber* spp.), and gilt sardine (*Sardinella aurita*). The interest about some of these species has been increasing as there is a new market for them; gilt sardine and mackerel, especially the first, are sold for tuna farming. A requirement for such sales is a high yield by fishing day, due to its low economic value. In the case of mackerel is mainly exported to Portugal.

The information of anchovy corresponds to the GSA01 (Northern Alborán Sea), but it is not known yet if this is a shared Mediterranean Moroccan and Algerian stock or a complete stock unit.

2.1.4 Western Mediterranean

A summary on this region is presented here below according to the work of Palomera *et al.* (2007)

Along the NW Mediterranean coast there are two relatively large (>60 km wide) continental shelves: the Gulf of Lions in the north and the Ebro shelf in the south. Between them the continental shelf is narrow, with a variable width always lower than 30 km. The slope is characterized by several submarine canyons, some of them almost reaching the coastline (3–4 km) (Figure 2.1.4.1). The circulation in the region is dominated by a south-westward current flowing along the edge of the continental shelf (Northern Current, NC). Associated with this current there is a typical shelf-slope front separating less saline in-shore waters from the waters of the open sea (Font *et al.*, 1988). This system exhibits considerable mesoscale activity that involves a certain spatio-temporal variability, especially in the upper layer during the stratified season (Wang *et al.*, 1988).

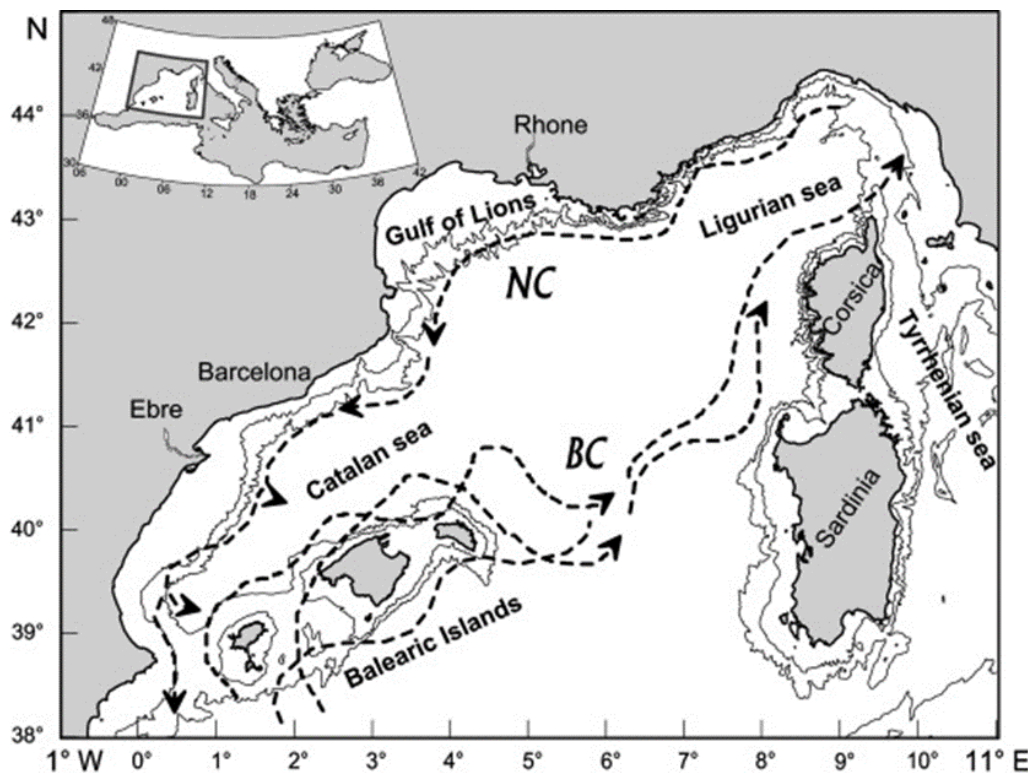
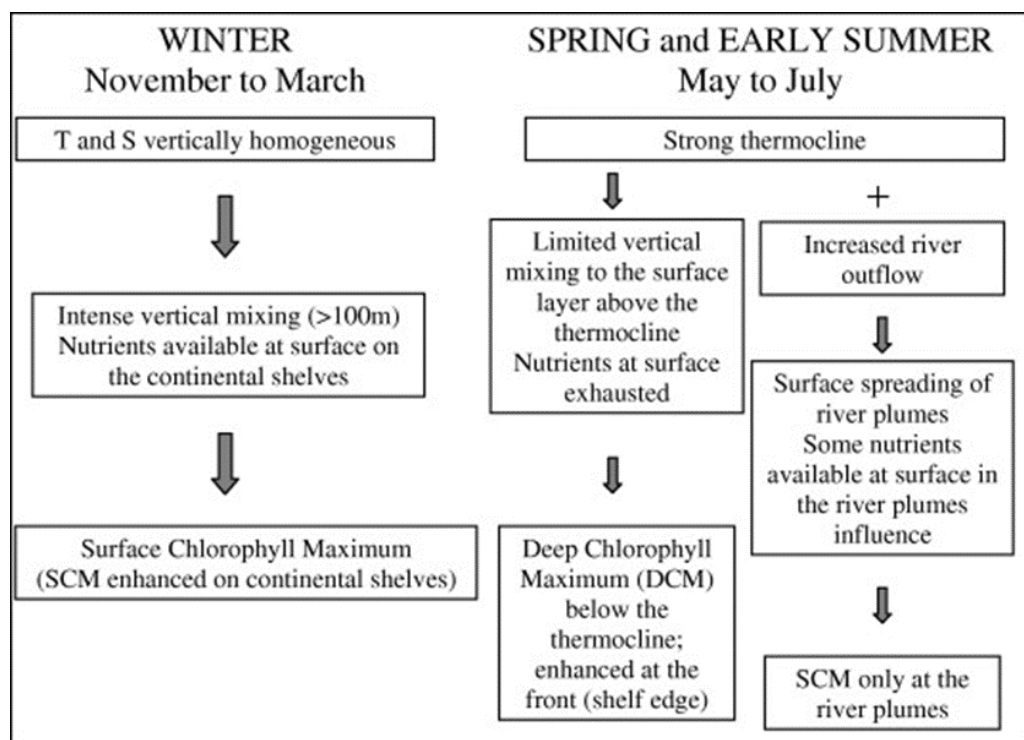


Figure 2.1.4.1. Map of the area showing the bathymetry (200 and 1000 m) and the paths of the main currents: Northern (NC) and Balearic (BC).

The coastal zones of the NW Mediterranean are often considered as oligotrophic. Surface waters only receive nutrients through vertical mixing, local upwelling, or terrestrial water discharges. Tides are very weak in the Mediterranean Sea and upwelling is episodic and restricted to very few locations. However, wind mixing is important in winter because the water column is homogeneous over the shelf. This effect is especially relevant over the wide continental shelves, not only due to their dimensions but also because they are located in the vicinity of the windiest areas. The most relevant terrestrial discharges are produced by the main rivers on the Catalan coast, the Rhône in the Gulf of Lions and the Ebro in the south, which collect the run-off from snow-covered mountain ranges (i.e. Alps and Pyrenees, respectively), wastewater from large cities and intensive agricultural and industrial activities. Other terrestrial inputs come from episodic rain storms that flow through otherwise almost dry rivers and from the discharge of nutrient-rich urban and

industrial effluents (Cruzado *et al.*, 2002). Surface waters over the Ebro shelf are an example of both types of nutrient supply: wind mixing and river run-off. Direct nutrient inputs from the Ebro outflow may account for 10–25% of the total nutrient content in the water column on the adjacent continental shelf (Salat *et al.*, 2002). These nutrients are introduced at the surface, thus becoming directly available for surface phytoplankton. This mechanism is especially relevant during the stratified season when wind mixing cannot reach the bottom to extract nutrients. The remaining nutrients in the water column (75–90%) are only available at the surface through winter mixing induced by the strong winds blowing over the area (Salat, 1996). In the absence of strong winds over a mixed water column or river run-off, phytoplankton production is restricted to the deep chlorophyll maximum (DCM) (Table 2.1.4.1) located at depths of 40–80 m (Margalef, 1968; Estrada, 1996). For example, the contribution of the DCM to the total chlorophyll in a shelf region with riverine inputs, like the Ebro region, varies from 30% to 100% depending on the season.

Table 2.1.4.1. Summary of the main productivity mechanisms in winter and summer in the North Western Mediterranean



Anchovy fishery in Western Mediterranean Sea-Northern Spain (GSA06)

Anchovy is the main target species of the purse-seine fleet in Northern Spain (GSA06) due to its high economic value. Catches in the period 1990–2015 has been highly variable, with a minimum of 2800 tonnes in 2007 and an average of 12000 tonnes. Higher catches occurred in the period 1990–1994, they were caught between 17 000 and 22 000 tonnes. Thereafter it has been continuously decreasing with three recoveries in 2002, 2009, and 2012. In 2014 and 2015 shows higher catches 16 000 t, a similar value to the one in 1990, but it is still not close to the peak of the landings occurred between 1991 and 1994. Years with higher landings are usually correlated with a successful and high recruitment period, while unsuccessful recruitment in a given year is correlated with a low level of landings.

The information of anchovy corresponds to the GSA06 (Northern Spain), but it is not known yet if this is a shared Mediterranean French stock or a complete stock unit. Studies of larvae transport from the Gulf of Lion to Spanish waters suggest that this is a shared stock.

2.1.5 Gulf of Lion

No new information was upload to this report and then interested readers are referred to WKARA (2009).

2.1.6 Tyrrhenian Sea

This area extends between parallels 38°N and 44°N and can be considered composed of very different subareas in river input and coastline structure, as well as continental shelf extension. On the basis of the mentioned aspects three main subareas could be characterized as follows (Figure 2.1.6.1):

- Area 1 extends along the south-eastern part of GSA 9 (North Tyrrhenian Sea). Such an area is characterized by wide continental shelf and moderately complex coastline morphology;
- Area 2 is located in the northern part of the GSA 10 along the coast of Campania. It is characterized by complex coastline morphology (mainly related to the presence of enclosed gulfs) and relatively wide continental shelf;
- Area 3 is the southern part of GSA 10. Very narrow continental shelf and moderately complex coastline morphology are evident features of this area. Another significant difference between the three identified areas is related to riverine input. In particular, area 1 and area 2 are characterized by a higher river run-off than area 3, where only very small rivers are present.

Analysis of the environmental dataset permitted identification, in two specific areas, of a pattern of variables driving enrichment processes and affecting on the habitat suitability of the two species. In the northern and central parts of the study area, both anchovy and sardine showed a marked preference for shallower areas characterized by lower salinity. In these areas, it has been highlighted a strong link between primary production, particulate organic carbon, distance from the mouth of the river, salinity and depth. A less clear picture was obtained for the southern part of the Tyrrhenian Sea, characterized by a narrow continental shelf, moderately complex coastline morphology and the presence of very small rivers. Most of the anchovy biomass was found to be located in enclosed areas (gulfs) under the influence of relatively small rivers. This finding, taking into account that the surveys were carried out during the anchovy spawning period, highlights for such species a positive effect of the interaction between coastal morphology and riverine input, probably favouring food supply and retention of spawning products (Bonanno *et al.*, 2016).

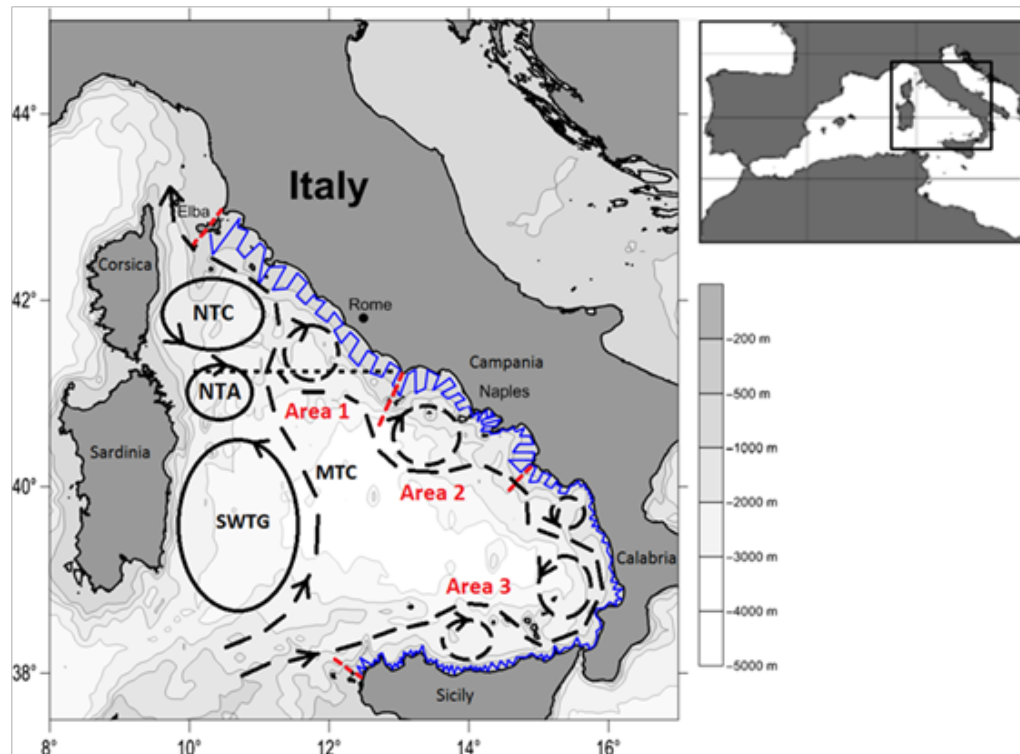


Figure 2.1.6.1. The study area in the Tyrrhenian Sea and the general survey design adopted during the four surveys (blue polyline). Areas 1, 2, and 3 are delimited by red dashed lines. Black dotted line represents the boundary between GSA 9 (northern area) and GSA 10 (southern area). The main surface circulation structures are shown: SWTG (South-Western Tyrrhenian Gyre), NTC (Northern Tyrrhenian Cyclone), NTA (Northern Tyrrhenian Anticyclone) and MTC (Middle Tyrrhenian Current). Continuous lines represent permanent circulation structures while dashed lines are used for semi-permanent/recurrent structure (Bonanno *et al.*, 2016).

General oceanographic features

Furthermore the South and Central Tyrrhenian Sea features one of the most complex structures in the seas around the Italian peninsula, due to its morphological and geophysical characteristics and water mass dynamics. The coastlines are generally very rugged and the island system is the most numerous in the Italian seas. The Tyrrhenian Sea is an active area with water mass movements, characterized by significant mesoscale dynamics (Vetrano *et al.*, 2010) (Figure 2.1.6.2). The waters can be classified into three main levels:

- The surface level, to a depth of 200 m, occupied by Modified Atlantic Water (MAW - AW in Figure 2.1.6.1), which flows with the Atlantic current from the Strait of Gibraltar and is modified enroute, increasing in salinity;
- The intermediate level, from a depth of around 200 to 700 m, is occupied by a mixture of intermediate waters (LIW, Levantine Intermediate Water) (Gasperini *et al.*, 2005) that flow from the Strait of Sicily;
- The deep level, occupied by Tyrrhenian Deep water (TDW), which flows out from the Sardinian Channel along the Sea of Sardinia.

The surface temperature (at -5 m) can vary from around 13°C in February to around 28°C in August, while the salinity ranges from 38.1 to 38.6 psu. The offshore waters are considered oligotrophic. At the coastal level, however, the areas in front of the river Volturno have eutrophic/mesotrophic features, whereas the strip in front of Naples and the mouth of the Sarno River shows phenomena of localized eutrophication. The Gulf of Salerno,

less affected by anthropic pressure, has mesotrophic characteristics, whereas oligotrophic conditions can be seen along the Cilentan coast.

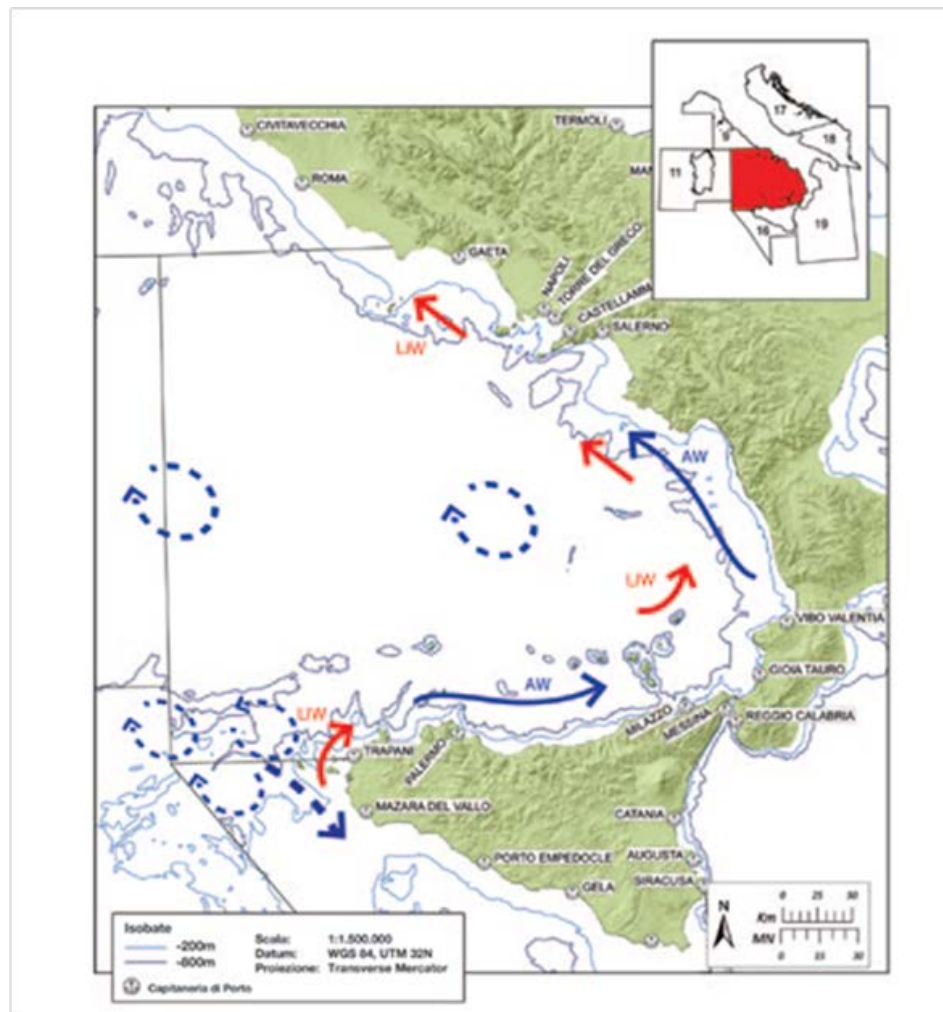


Figure 2.1.6.2. Circulation of surface (AW) and intermediate (LIW) currents; AW: waters of Atlantic origin (blue); LIW: waters of Levantine origin (red).

Taking into account the geo-morphology, circulate water, temperature and trophic level of GSA 10 the main anchovy population and fishery are localized in the north part (Gulf of Gaeta, Gulf of Naples and Gulf of Salerno), in the Gulf of S. Eufemia along the Calabrian coast and in the north part of Sicily mostly in the area around Palermo. The spawning season extends from late spring (March) to early autumn (September) with a peak spawning in July. Length at first sexual maturity (L_{50}) was estimated 10.6 cm (maturity range L_{25} – L_{75} = 9.95–11.25) for female and 9.9 cm (maturity range L_{25} – L_{75} = 8.9–10.8) (Spedicato *et al.*, 2015). Growth parameters obtained by direct aging were: L_{∞} = 18.92 cm, k = 0.245 year⁻¹ and t_0 = -2.037 year for sex combined (Spedicato *et al.*, 2015).

2.1.7 Strait of Sicily

The Strait of Sicily (Figure 2.1.7.1) is an upwelling area with complex circulation patterns mainly determined by the motion of the Atlantic Ionian Stream. The latter produces a cyclonic vortex over Adventure Bank causing the formation of a permanent upwelling along the south coast of Sicily (Bonanno *et al.*, 2014). Wind-induced upwelling events often occurring in the coastal area reinforces the permanent upwelling process (Patti *et al.*,

2010). The intensity of the upwelling processes causes high interannual variability of primary production that also affects the interannual variability of small pelagic fish. In such area *Engraulis encrasicolus* stocks are highly variable in recruitment, biomass and spatial distribution with biomass values ranging from 3000 to 20 000 t (Bonanno *et al.*, 2014). Changes in habitat conditions may influence both the survival of the early life stages and the adult stages. Detailed studies on this species in this area, highlighted the importance of environmental processes for the growth, reproductive biology, spatial distribution and habitat selection (Basilone *et al.*, 2004, 2006, 2013, 2015; Bonanno *et al.*, 2014; Barra *et al.*, 2016).

All these habitat variability may be responsible not only of the growth pattern on Otoliths but also on the morphological aspect and rings readability in the Strait of Sicily. Although further comparative studies have to be addressed to such a topic to receive more robust evidences.

The anchovy spawning season, as defined by GSI, usually began in March or April (although in some year it began in June), peaked in June and July, and ended by mid-September or October. The intra-annual variation in the reproductive cycle was clearly synchronized with water temperature, as the onset of the reproductive cycle was associated with water warming and its end with water cooling (Basilone *et al.*, 2006).

There were also interannual GSI variations during the spawning period for both female and male specimens. GSI during the spawning period was significantly correlated with mean chlorophyll concentration from October of the previous year to May (Basilone *et al.*, 2006). Chlorophyll concentration explained 92% of the total variance observed in female GSI and 55% of that in male GSI (Basilone *et al.*, 2006).

There are intra-annual variations linked to timing of the phytoplankton bloom. Monthly condition factor had a significant correlation with monthly chlorophyll concentration, with a four month lag. Length-weight relationships corroborate the findings obtained from analyses of variability of condition factor. Changes in total anchovy weight for a given body length are mainly due to changes in the chlorophyll concentration (Basilone *et al.*, 2006).

Maturity in some specimens was evident at 9.4 cm (females and males), and all individuals longer than 13.5 cm. were mature. Mean L_{50} values for the whole period were 11.27 cm \pm 0.09 for males and 11.24 cm \pm 0.09 for females, although a more accurate estimation could be obtained by using microscopic and macroscopic gonad examination (Basilone *et al.*, 2006).

The von Bertalanffy growth model and back-calculation analyses were applied to estimate anchovy growth parameters using the FiSAT program. There were not significant differences between males and females, pooled mean parameters were L_{∞} = 18.6 cm, k = 0.29 per year and t_0 = -1.81 years (Basilone *et al.*, 2004).

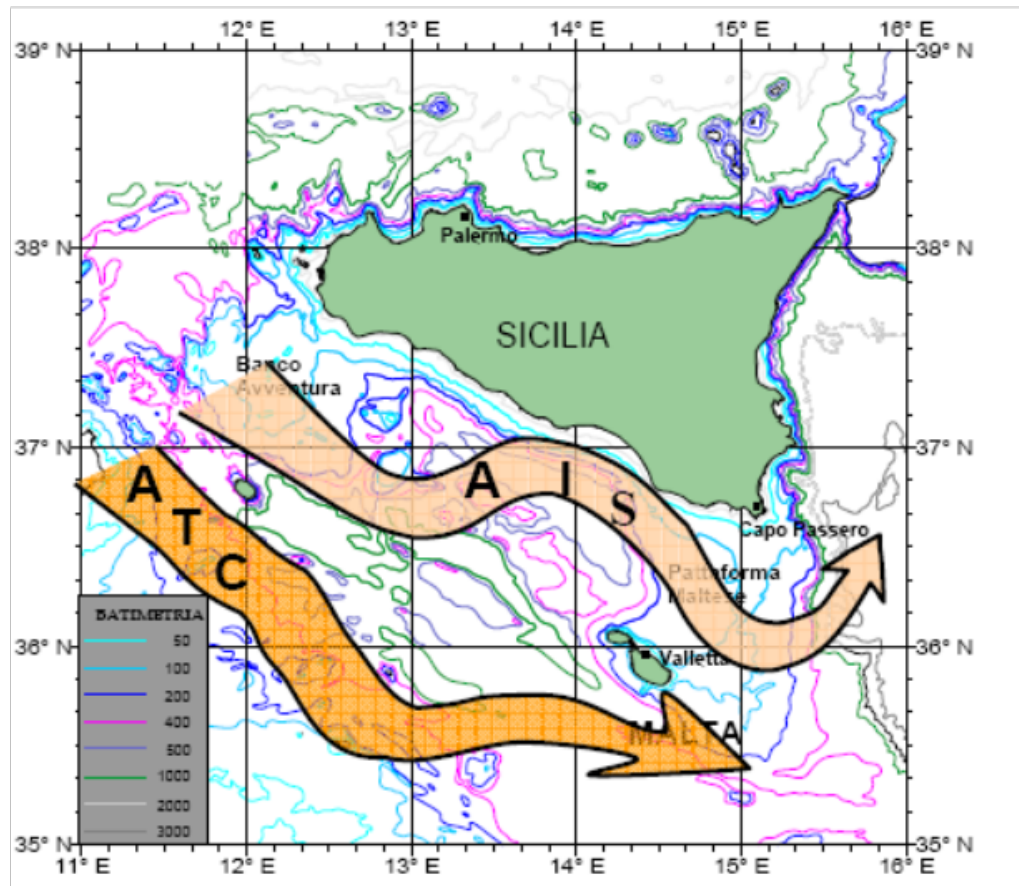


Figure 2.1.7.1 Main surface circulation patterns in the Strait of Sicily with the two main branch coming from the Modified Atlantic Water (MAW) that in this area split into the Atlantic Tunisian Current (ATC) and the Atlantic Ionian Stream (AIS).

2.1.8 Ionian Sea

Anchovy in GSA19 (Western Ionian Sea) is mostly captured by purse and small-scale driftnet (STECF, 2013). Regarding the von Bertalanffy growth parameters for both females and males are: $L_{\infty} = 174.13$ mm, $k = 0.31$ years⁻¹, and $t_0 = -1.76$ year for the GSA19 (Intini *et al.*, 2010), $L_{\infty} = 175$ mm, $k = 0.509$ years⁻¹ and $t_0 = -0.888$ (Machias *et al.*, 2000a) in the GSA20 (Central Aegean and Eastern Ionian Seas). In both case (GSA19 and GSA20) the samples utilized come from experimental pelagic trawl and onboard sampling.

The spawning period in Ionian Sea is included between May to September with a peak in June (Somarakis, 1999). Food (mesozooplankton) availability seems to be linked with the reproductive effort, indeed the energy for reproduction derives primarily from the food intake (Somarakis *et al.*, 2006 in Palomares *et al.*, 2006).

The vertical and horizontal distribution and the biomass in the coastal areas of the Central Aegean and Ionian Seas have been studied during 1995–2001 (Machias *et al.*, 2001; Giannoulaki *et al.*, 2003). Acoustics and DEPM methods have been applied for the estimation of biomass in the same areas in June 1999. The abundance estimates ranged between 11 000 t and 15 000 t. Anchovy reach maturity at approximately 105 mm total length, at first year of life in Central Aegean and Ionian seas (Palomares *et al.*, 2006).

Studies regarding genetic structuring for European anchovy in the eastern Mediterranean and the Black Sea provide consistent evidence that the anchovy stocks do not form a genetically homogeneous population (Machias *et al.*, 2000). Two different genetic stocks of anchovy are currently recognized in Greek waters: the eastern stock (Aegean type) and

the Western stock (Ionian type) (Somarakis *et al.*, 2005). Ionian Sea is inhabited by small stocks of anchovy with patchy distributions (Somarakis *et al.*, 2004). Some marked inter-regional or interannual differences in anchovy reproductive parameters have been observed in the more southern Mediterranean areas, such in GSA20. These areas are largely oligotrophic, receiving the influence of only small rivers or wind-driven, but highly variable, upwelling. A remarkable difference in batch fecundity was found between the central Aegean and Ionian stocks in 1999. However, the interplay of fecundity and spawning frequency seemed eventually linked to condition reproductive effort; because the estimates of spawning frequency showed an opposing trend and resulting values for daily specific fecundity were almost equal for the two seas (about 18 eggs g⁻¹ of reproducing stock) (Table 2.1.8.1)

Table. 2.1.8.1 Adult parameter estimates of European anchovy DEPM applications in Ionian Sea. (Table from Somarakis *et al.*, 2004). R is the sex ratio by weight, W the average weight of mature females (g), F the batch fecundity, S the spawning fraction, RF the relative fecundity (RF = F/W, eggs g⁻¹), and DSF is the daily specific fecundity, the number of eggs produced per g weight of the population (DSF = FSR/W). CV in parentheses.

R	W	F	S	RF	DSF
0.53 (0.07)	15.6 (0.05)	9.428 (0.08)	0.06 (0.26)	604	19

2.1.9 Adriatic Sea

No new information was uploaded to this report and then interested readers are referred to WKARA (2009)

2.1.10 Aegean Sea

The Aegean Sea is located between the Greek and Anatolian peninsulas in the Eastern Mediterranean Sea. Its northern portion it is connected to the Marmara Sea and Black Sea by the Dardanelles and Bosphorus. Its coastline is characterized by rocky coasts interrupted by large portions of sandy beaches and in its northern portion it presents a large number of wetlands and lagoons. The seabed sediment in the Aegean Sea is mainly muddy; *Posidonia oceanica* meadows are predominant in shallower waters up to a depth of 25 m (Orfanidis *et al.*, 2000). The bathymetry of the northern part consists of a wide continental shelf, which slopes gently to a depth of around 35–40 m up to the north coast of the main islands of Thasos and Samothrace, going down to 100–200 m to the southern portion of these islands. On the contrary Mount Athos, in the Chalcidice Peninsula, presents very narrow continental shelf and the waters drop rapidly to 500–600 m depth (Perrissoratis *et al.*, 1984). Depth increases in its central and southern portion up to 3500 m eastern of Crete. In the central Aegean Sea many groups of islands are present so that in the ancient times the name of Archipelagos was given to the entire basin. The tidal activity in the entire area varies up to 30 cm and can be considered insignificant.

The waters of the Aegean Sea are influenced by the presence of numerous rivers in its northern portion, like Axios, Strimonas, Nestos, and Evros (Sylaios *et al.*, 2005) and by the water entering from the Black Sea especially during winter and spring (Poulos *et al.*, 1997). These fresh and cold waters are pushed up to the north coast of the mainland by the Earth's clockwise rotational movement, where they proceed along the coast in an anti-clockwise movement. Due to the presence of Thasos, two anticyclone gyres are created, on both the eastern and western sides of the island (Somarakis *et al.*, 2002). When the water of the main stream reaches the Chalcidice Peninsula, it turns southward and eastward. It concludes its tour north of Limnos, returning back to the Turkish coast from a south-north direction (Kontoyiannis *et al.*, 2003; Kourafalou, 2007). Thanks to both the

Black Sea and river water influxes, the northern part of the Aegean Sea is rich in nutrients and represents typical eutrophic conditions (Somarakis *et al.*, 2002; Somarakis and Nikolioudaki, 2007). The physical, chemical and biological parameters, such as particulate organic carbon (POC), particulate organic nitrogen (PON), chlorophyll *a* and organic matter concentrations, are some of the highest in all the Eastern Mediterranean Sea (Syllaios *et al.*, 2005; Stamatis *et al.*, 2006). The eastern part of the Aegean Sea south of the Dardanelles straits is influenced by Levantine's warm and saline waters; the presence of such different water masses creates strong temperature and salinity contrasts between the N-NW cold and freshwaters and the S-SE warm and saline ones. The surface salinity values increase proceeding southward through the Aegean Sea (Nittis and Perivoliotis, 2002).

Phytoplankton in the Aegean Sea normally has a very important peak around February-March, especially in the northern portion, and a secondary peak during November-December. Spring peak is due to the strong rainfall during the previous winter and early spring that drives a large amount of nutrients from the rivers of the north coast to the sea (Boskidis *et al.*, 2007).

Zooplankton biomass is also considered one of the highest in the Eastern Mediterranean with a higher percentage of small-sized short-lived pelagic species such as *Engraulis encrasicolus* and *Sardina pilchardus* (Giannoulaki *et al.*, 2005). The higher concentration of ichthyoplankton is recorded in between Thasos and Samothrace Islands and in between Thasos and Mount Athos where two small anticyclonic gyres produce a local upwelling (Somarakis *et al.*, 2002). The major spawning and nursing grounds of anchovy in the Aegean Sea and in the entire Greece are located in the North Aegean Sea (Giannoulaki *et al.*, 2008). Mature female anchovies are caught from March to September but the highest percentages (75% and 76%) have been recorded in August and September, indicating the peak spawning time; the proportion of immature individuals in October and November have been recorded as 81% and 100%, respectively (Kallianiotis *et al.*, 2002). Anchovy reach maturity at approximately 105 mm total length, at the completion of the first year of life. The reproductive effort of spawning anchovy seems to be closely associated with adult prey fields (meso-zooplankton) and the energy allocated to reproduction derives primarily from the food intake (Somarakis *et al.*, 2005). von Bertalanffy growth parameters estimated by several authors from samples collected from landings, experimental trawling and on board the commercial fishing fleet, indicate for the North Aegean Sea K values from 0.280 to 0.860 year⁻¹, L_{∞} from 191 to 219 mm and t_0 from -2.480 to -1.276, while for the Central and Southern Aegean K ranges from 0.314 to 0.509, L_{∞} from 175 to 191 mm and t_0 from -1.839 to -0.888 (Somarakis *et al.*, 2005). The direct biomass estimates for anchovy through acoustic and/or egg surveys ranged between 40 000 and 45 000 t in the North Aegean and between 11 000 and 15 000 t in the Central Aegean Sea (Somarakis *et al.*, 2005).

In the Aegean Sea anchovy is fished with purse-seines from the 1 March to the 15 December. The conventional birth date is assumed to be the 1 June. Spawning season goes from spring to autumn with a peak in June-July. Season of recruitment-at-age 0 is assumed to be in March.

The European anchovy in the northern part of the GSA 22 was studied since several years at the Fisheries Research Institute of Kavala using market and deck sampling (AA.VV, 2013). During the years 2000–2001 the length of the anchovies sampled ranged between 100–180 mm with a peak at 130–140 mm. The length at the first reproduction was estimated at 103 mm for females and 106 mm for males. The reproductive season was for both sexes from March to September, with a peak on August and September. Sex ratio (M/F) was 0.88 in 2000 and 0.87 in 2001. von Bertalanffy equation gave a back calculated

length for females of 120.42 at age 1 and 142.81 at age 2, while for males of 116.60 at age 1 and 128.56 at age 2. The population was composed of four age groups: 1, 2, 3, and 4 years. Most abundant was age 2 (67.9%) followed by age 3 (18.9%). Length-weight regression showed b values higher than 3, indicating an allometric growth. Data from 2013 showed a length composition ranging from 55 to 170 mm. The 80% of the individuals had length between 100 and 129 mm. Length-weight relationship was considered almost isometric ($b = 3.007022$). Sex ratio was in favour of males for individuals up to 109 mm and of females for the larger individuals. Sexual maturity was major than 50% for individual from 115 mm. The population was composed of four age groups: 0, 1, 2, and 3 years. The majority of the individuals were age 1 (61.17%) followed by individuals of classes 0 (21.18%) and 2 (17.32%). Individuals of age 3 were extremely rare (0.33%).

2.1.11 Tunisian area

In Tunisia, the European anchovy is of a great economic importance. Its biomass estimated by hydroacoustic surveys averaged 6000 tonnes (Gaamour *et al.*, 2005). It is caught along the coasts all year-round with a maximum of landing recorded in July (National Statistics).

Anchovy is a gonochoric species characterized by a balance of numerical proportions of both sexes. The first maturity occurred during the first year of life for the anchovy. Females and males reach their first sexual maturity at an equivalent size; however the size of first sexual maturity varies from area to another in Tunisian coasts. Indeed, the largest size of first sexual maturity is observed in the North area at 8 cm fork length while the smallest is recorded in the South region at fork length of about 6.2 cm. For the anchovy, the spawning period corresponds to a single annual sexual cycle with an average reproduction period lasting from April to October (Gaamour *et al.*, 2004b; Gaamour *et al.*, 2005; Khemiri, 2006). An average date of birth was established on 15 July (peak of spawning females and males). The analysis of the distribution of the back-calculated hatch dates showed that the recruits of the anchovies came mostly from the spawning of June. The condition of the anchovy goes through two periods during an annual cycle; a first spring-summer period (April–September) of good condition coincides with the breeding season. It is characterized by the fattening of the fish which culminates in summer and a second period of bad condition marked by an anchovy slimming at the end of the breeding season. This period begins in October and reaches minimum values in February (Khemiri and Gaamour, 2009).

Analysis of the otolith marginal zone indicated that there is one only cycle of growth per year with a slowing of growth from November/December to March followed by one period of active growth. This growth cycle is mainly synchronized by the sea temperature, reproduction and the food availability (Khemiri, 2006; Gaamour *et al.*, 2005; Khemiri *et al.*, 2007).

In Tunisian waters, anchovy showed a positive allometry (Khemiri and Gaamour, 2009; Khemiri, 2006). It has longevity of at least four years. It had a fast growth during its first year of life; indeed the growth was almost achieved during the first year of life for the anchovy. No growth differences were observed between males and females. However, for the same area, a growth difference existed between populations of anchovy from the open sea and those from more coastal areas. These differences might be related to variations of the environmental conditions but genetic differences could also be responsible for growth differences (Khemiri, 2006).

Ecosystem considerations for anchovy populations

The Tunisian coasts appear as a mosaic of different structures that are organized along a coast-deep water and north-south gradient. The main structural elements of the coast are hydrodynamics, water temperature, salinity, planktonic entity and bathymetry (Azouz, 1973; Brandhorst, 1973; Sammari and Gana, 1995). This structuring made it possible to delimit the four following geographical regions that would have different habitats for small pelagics:

- North (GSA12): 36°58'N 8°40'E-37°10'N 10°16'E: It has a rugged relief that can have a beneficial effect on the fertilization of the environment. It is under the direct influence of the Atlantic vein characterized by low temperature, low salinity and high concentrations of nutrient salts and planktonic species.
- Gulf of Tunis (GSA12): 37°10'N 10°16'E-37°04'N 11°02'E: The continental shelf is fairly wide and relatively shallow. The coastal zone of the gulf is under the influence of a hot and hyposaline current coming from the littoral sector. The Atlantic current is perceptible only in winter and spring. The abundance and specific composition of the Copepod community follows a coast-to-wide gradient.
- East (GSA13): 37°04'N 11°02'E-35°N11°E: It includes the Gulf of Hammamet. It is a transition zone between the eastern and western basin. The relief features rugged areas and areas with a fairly wide and fairly flat continental shelf. The temperature of the water and the salinity resemble those of the northern region. In winter, the column of water is subjected to a vertical mixing and in summer, an upwelling would be established favouring the enrichment of the environment.
- South Region (GSA14): 37°04'N 11°02'E-35°N11°E: It includes the Gulf of Gabes. It is characterized by a broad and shallow continental shelf. This relief is responsible for the strong seasonal variations of the salinity and temperature of the water and the formation of thermal fronts in winter and summer. Phytoplankton primary production is very low.

2.1.12 North of Morocco

Anchovy has traditionally been the target species of the Spanish purse-seine fleet operating in the Atlantic waters off North Morocco, developed in the framework of Fisheries Partnership Agreements (FPAs) between the EU and the Kingdom of Morocco. The current FPA between the EU and Morocco (Council Regulation (EC) N° 764/2006 of 22 May 2006) entered into force on 28 February 2007 for a period of four years and it has been tacitly renewed twice. [The current Protocol](#) to this Agreement (OJ L328, 2-39, 7-12-2013) entered into force on 15 July 2014 following the completion of the internal ratification procedures by Morocco. The activity of this fleet has been limited to the northern sector of the region above latitude 34°18'00".

The Moroccan stock of anchovy is assessed in an annual basis by the Fishery Committee for the Eastern Central Atlantic (CECAF), in the FAO Working Group on the Assessment of Small Pelagic Fish off Northwest Africa. In the absence of studies on the stock identity of this species, this Working Group (FAO, 2015) considers anchovy (*Engraulis encrasicolus*) in the Zone North (35°45'-32°N), Zone A (32°-29°N) and Zone B (29°-26°N) as belonging to a single stock (Figure 2.1.12.1) (FAO, 2015).

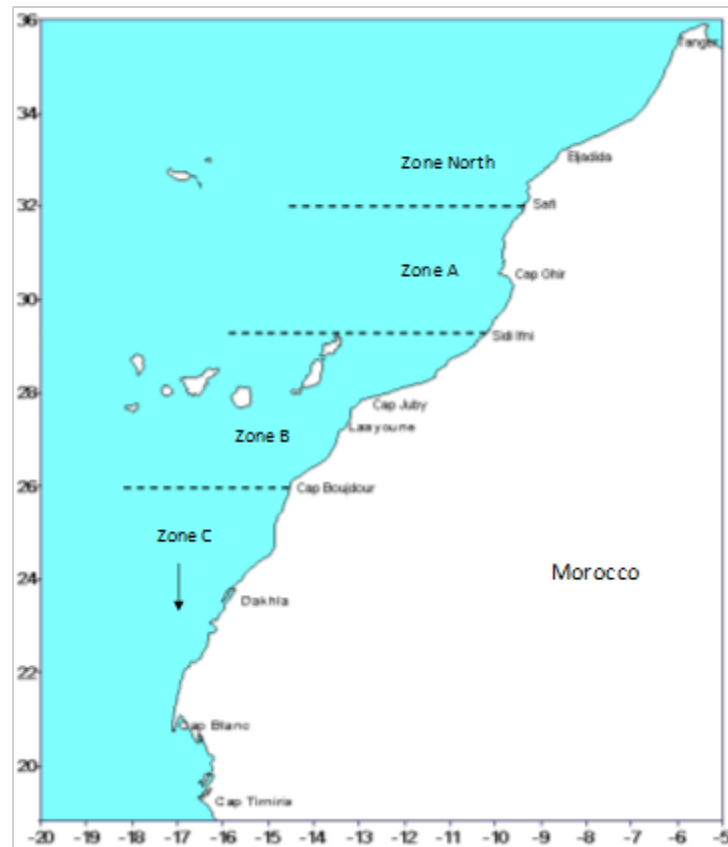


Figure 2.1.12.1. Moroccan fishing zones.

The length frequencies for anchovy caught by the Spanish fleet in Zone North range between 7 and 19 cm, with a mode around 15 cm. These sizes are bigger than the ones recorded by the Moroccan fleet (FAO, 2015), due to the restriction of the fishing area for the Spanish fleet, imposed by the Protocol, to deeper zones where the recruits are not abundant. The spawning season in the northeastern Moroccan area, where the Spanish fleet operates, may be very similar to the observed for the species in the Gulf of Cadiz, that is, from March-April to November (Ramos *et al.*, 2008).

The main spawning area was located in Cape Ghir-Sidi Ifni (30°38' N), with possible season displacements. A secondary spawning area exists between Punta Ajefnir and Cape Juby (27°56' N) (Ettahiri, 1996). The zone between Cape Draa (28°45' N) and Cape Juby, an area with a shallow and wide shelf and important primary and secondary production, also constitutes an important spawning zone.

2.2 Summary information by Stocks/areas of European Anchovy

A questionnaire was distributed among anchovy ageing institutes before the exchange 2014 and WKARA2 to get an overview of information of anchovy stocks in different areas (biology, fisheries, etc.) from Atlantic area and Mediterranean Sea (Tables 2.2.1 and 2.2.2) and the MAP (Figure 2.2.1).

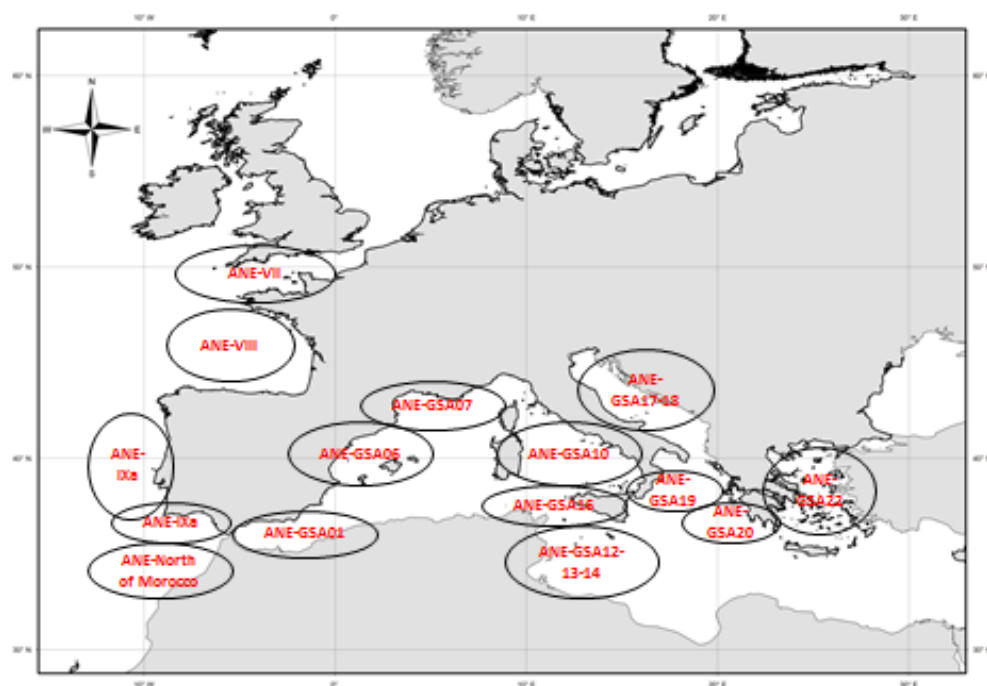


Figure 2.2.1 Map of the different areas for which otoliths examined in the current workshop

Table 2.2.1: Summary of information of Anchovy stocks/areas in Atlantic areas.

Anchovy Stock	Area	Analytical Stock Assessment?	Organization/ Working Group	Countries involved/Institutes	Conventional birth dates	Season of fishery	Peak of catch	Spawning Season	Spawning peak	Typical Length range	Typical Age range	Season of recruitment at age 0
English Channel	ICES Subarea VII	No	ICES WGHANSA	England and France/CEFAS, IFREMER	1st of January	All year	-	-	-	-	-	-
Bay of Biscay	ICES Subarea VIII	Yes	ICES WGHANSA	France and Spain/IFREMER, AZTI and IEO	1 st of January	March-November	April-June for Spain; June-September for France	April-August	May-June	11-18 cm	1-3 years	Autumn (September-October)
Division IXa	ICES Division IXa	Not yet (trend-based qualitative assessment)	ICES WGHANSA	Portugal and Spain/IPMA and IEO	1 st of January	February-November for Ixa South, Spain; Occasional for Portugal in IXA Cnorth, Csouth and South, and for Spain in Ixa North	June-July (IXA South, Spain); No peak for the rest of the areas.	April-November for Ixa South	June-July for Ixa South;	9-15 cm (Ixa South); 11-17 cm (Ixa North)	0-2 (Ixa South); 0-3 (IXA CN-CS-S); 1-3 (Ixa North)	Autumn (September-November)
North Atlantic area (North of 34°18'00")	Morocco EZZ	Yes	FAO WG on the Assessment of Small Pelagic Fish off	Morocco and Spain/INRH and IEO	1 st of January	April-November/January	Irregular pattern of catches during the last years.	March-April to November	Summer, from June to September	9-19 cm	No data	No data

Table 2.2.2: Summary of information of Anchovy stocks/areas in Mediterranean areas.

Anchovy Stock	Area	Analytical Stock Assessment?	Organization/ Working Group	Countries involved/Institutes	Conventional birth dates	Season of fishery	Peak of catch	Spawning Season	Spawning peak	Typical Length range	Typical Age range	Season of recruitment at age 0
GSA01	Northern Alboran Sea	Yes	CGPM (WG small pelagic species)	Spain/IEO	1 st of July	All year	Summer/Autumn	May-October	July	11-16 cm	0-1 years	Autumn
GSA06	Western Mediterranean	Yes	CGPM (WG small pelagic species)	Spain/IEO	1 st of July	All year	Summer	May-October	July	11-16 cm	0-1 years	Autumn
GSA07	Gulf of Lion	Yes	CGPM (WG small pelagic species)	France/IFREMER	1st of January	All year	July	May-August	July	7-16 cm	0-3 years	Autumn
GSA10	Southern Tyrrhenian	No	CGPM (WG small pelagic species)	Italy/COISPA	1 st of July	Summer-Autumn	July	Spring	May-June	9-15 cm	1-3 years	Summer and early autumn
GSA12	North of Tunisia	No	CGPM (WG small pelagic species)	Tunisia/INSTM	15 of July	March to August	April	April to October	July	8-16 cm	1-3 years	October
GSA13	Gulf of Hammamet	No	CGPM (WG small pelagic species)	Tunisia/INSTM	15 of July	March to August	July	April to October	July	8-16 cm	1-3 years	October
GSA14	Gulf of Gabes	No	CGPM (WG small pelagic species)	Tunisia/INSTM	15 of July	April to October	April and October	April to October	July	8-16 cm	1-3 years	October

Table 2.2.2: cont.¹

Anchovy Stock	Area	Analytical Stock Assessment?	Organization/ Working Group	Countries involved/Institutes	Conventional birth dates	Season of fishery	Peak of catch	Spawning Season	Spawning peak	Typical Length range	Typical Age range	Season of recruitment at age 0
GSA16	Strait of Sicily	Yes	CGPM (WG small pelagic species)	Italy/IAMC-CNR	1 st of July	All year	Summer	May-October	July-August	8-17 cm	0-3 years	Autumn (September-December)
GSA17&18	Adriatic Sea	Yes	CGPM (WG small pelagic species)	Croatia and Italy/IOF and ISMAR	1st of June	All year	Spring/Summer	April - October	April - July	6-17 cm	0-3 years	Autumn
GSA19	Western Ionian	Yes	STECF-14-08	Italy/COISPA	1 st of July	Summer-Autumn	June	Spring	May-June	9-15 cm	1-3 years	Summer and early autumn
GSA20	Eastern Ionian	Yes	CGPM (WG small pelagic species)	Greece/ELGO and HCMR	1st of June (ELGO)/ 1st of July (HCMR)	March to November	July	Spring-Autumn	June-July	9-15 cm	1-2 years	March
GSA22	Aegean Sea	Yes	CGPM (WG small pelagic species)	Greece/ELGO and HCMR	1st of June (ELGO)/ 1st of July (HCMR)	March to November	July	Spring-Autumn	June-July	9-15 cm	1-2 years	March

¹ After this workshop ELGO has decided to move the conventional birthdate to 1 July as well.

3 Review information on anchovy age estimations, otolith exchanges, workshops and validation work (ToR a)

3.1 Background information on age determination, Otolith exchanges and Workshops on Age Reading of European Anchovy

European anchovy (*Engraulis encrasicolus*) is a small pelagic species of high commercial importance in European waters, in both the Atlantic and the Mediterranean Sea, and is assessed in most of the stocks that are distributed in these areas. The assessments are conducted within the framework of ICES for stocks in the Atlantic area (ICES, 2014) and in the GFCM for stocks in the Mediterranean Sea (GFCM-FAO, 2014).

Ages reading on anchovy are important input data for the assessment and carried out by number of laboratories using international ageing criteria. There is an international age reading protocol and a consensual age reading criteria for Atlantic and Mediterranean areas from the last Workshop on Anchovy age reading in 2009 (ICES, 2009).

In the past, since the 1990s, exchanges, workshops and cross-checking of the procedures for age determination of European anchovy otoliths in Atlantic areas have been made in the Bay of Biscay (Astudillo *et al.*, 1990; Villamor and Uriarte, 1996; Uriarte, 2002a; Uriarte *et al.*, 2002, 2006 and 2007) and in the Gulf of Cadiz (Garcia, 1998; Uriarte *et al.*, 2002). However, no proper exchanges or workshops on reading procedures of European anchovy otoliths had been held in Mediterranean areas until 2009 (ICES, 2009).

Since 2009, there have been two exchanges and one workshop on Anchovy otoliths taking into accounts the Atlantic and the Mediterranean areas together (Table 3.1.1). 2008 PGCCDBS (ICES, 2008) recommends the realization of first otolith exchange and workshop of anchovy between the Atlantic and Mediterranean areas together (ICES, 2009; Villamor *et al.*, 2009). In 2014, the PGCCDBS (ICES, 2014a) identified the need of a full-scale European Anchovy otolith exchange to take place in 2014.

The overall result of the last exchange exercise in 2014 (Villamor and Uriarte, 2015) was that there were significant variations in anchovy age estimates between readers and areas. Low precision, and large relative biases between readers and areas were found. Most of the anchovy otoliths were not well classified by many of the readers during the 2014 exchange. In general, the results of the expert group improved compared to those of intermediate and training group in all areas. The results of the area readers group were more consistent than between the other groups of readers (including expert group). This may mean that there are different reading criteria between areas, so that when comparing only the readers in their expertise area they are more consistent because they all follow the same reading criteria. Analysis only done with the area readers group shows a higher overall agreement and low CV for Aegean Sea and Bay of Biscay readers. Possibly the success of the readers of the Bay of Biscay, compared with the other sets, is because exchanges and workshops have been conducted since 1990 in this area, and there are sufficient criteria for the interpretation of anchovy otoliths. In the case of Aegean readers which show a great accuracy of its readings, both readers are of the same institute and therefore would have very consistent criteria. For more information see Section 7.1 and Annex 4 of this report and in Villamor and Uriarte (2015).

2015 WGBIOP (ICES, 2015) recommends the realization of a Workshop on Age Reading of Anchovy for all European countries in 2016 (WKARA2), in order to ascertain the current level of precision among institutes and the difficulties that the age reading of anchovy otoliths present. This report represents the results of this workshop.

Table 3.1.1. Summary of the anchovy otolith workshops and exchanges

WK/Ex- change	Area	Mode of prepara- tion	% Agreement	CV
			(All read- ers/area readers)	
Ex- change 2014	English Chan- nel-7	Whole otolith, in resin (only images)	66.7/-	127.6
	Bay of Biscay-8		74.3/90.9	45.1/11.4
	Division 9a		68.5/75.7	49.1/33.0
	Albora Sea- GSA01		58.9/-	58.7/-
	Western Medi- terranean- GSA06		60.9/-	49.9/-
	Gulf of Lion - GSA07		73.4/-	31.3/-
	Southern Thyrranian- GSA10		62.9/67.3	67.2/58.1
	Strait of Sicily- GSA16		58.5/85.6	78.7/11.2
	Western Ionian -GSA19		61.9/73.5	60.9/55.3
	Aegean Sea- GSA22		70.0/97.1	55.7/6.7
WKARA 2009	Bay of Biscay	Whole otolith, in resin (otoliths and images)	86.2/92.5	41.4/8.1
	Alboran Sea		75	66.4
	Strait of Sicily		61.9	67.3
Ex- change 2009	Bay of Biscay		72.2/ 88.8	84.5/12.9
	Gulf of Cadiz 9a		58.3	68.1
	North of Mo- rocco		60.7	99.8
	Alboran Sea		64.1	61.6
	North Adriatic Sea		55.6	72.2
	Gulf of Lion		71.5	37.4
	North Adriatic Sea		60.3	63.3

As we have seen above in this section, considerable efforts are made by international committees to standardize the anchovy age interpretation in European waters. During these exchanges and workshops, the samples used are rarely validated therefore the « true age » is not known. In this way, the calibrations demonstrate the precision of age estimation between readers but not the accuracy (Secor *et al.*, 1995; Panfili *et al.*, 2002).

3.2 Validation studies

Several methods exist for validation of age readings of calcified structures (Campana, 2001). A summary of age validation methods used for anchovy in European waters is shown in Table 3.2.1.

Validation of anchovy annual ageing method is achieved following the marginal otolith structure development throughout the year for validating the periodicity of annual growth increment formation in several areas: Bay of Biscay, Gulf of Cadiz, Alboran Sea, NW Mediterranean Sea, Strait of Sicily, and North Adriatic Sea (Pertierra, 1987; Morales-Nin and Pertierra, 1990; Uriarte, 2002b; Uriarte *et al.*, 2002; Giraldez and Torres, 2009; Donato and La Mesa, 2009; Millan and Tornero, 2009; Uriarte *et al.*, 2016) (Table 3.2.1). In all cases the qualitative method was used (Panfili *et al.*, 2002). The major pattern shows that the true annual ring is formed in winter each year (hyaline edge) with a maximum growth in summer (opaque edge), but starting of the opaque edge during spring changes with ages, being remarkably sooner at age 1 than at older ages. Other rings may be present throughout the year mainly in summer (checks or false rings) as noted in several places (see next section). However, this general pattern shows a great interannual variability and evidence the complexity for age determination in this species as well as the difficulties in the recognition of the expected general growth pattern of the otolith through the year. It has been recognized in previous studies that a very large proportion of the variability observed in the anchovy growth between areas (and within the same area as well) could be explained by changes in the habitat conditions, namely chlorophyll concentration and temperature (e.g. Basilone *et al.*, 2004; Giraldez and Torres, 2009), although miss interpretation of the actual otolith growth pattern might also be behind of part of such apparent variability, as it was highlighted during the workshop.

The age estimation criteria of Bay of Biscay anchovy were also corroborated (or indirectly validated) by tracking year-classes abundance indices 1982–2013 in research surveys in the Bay of Biscay (Uriarte, 2002; Uriarte *et al.*, 2002; Uriarte *et al.*, 2016).

In the NW Mediterranean Sea, length frequency analysis method was applied to corroborate the otolith interpretation and growth model parameters of anchovy (Pertierra, 1987; Morales-Nin and Pertierra, 1990).

Based on different daily growth studies, the formation of the first annulus was validated and the position of the first false ring or check was corroborated in anchovy in the Bay of Biscay (ICES, 2013). In addition, Annual increment deposition in the otoliths of young-of-the-year European anchovy was validated by examination of micro-increments (Aldanondo *et al.*, 2016). Early anchovy juveniles were maintained in captivity from October 2012 until April 2013 and the first annulus was validated using daily increments counts. According to that, the first opaque band is completed in October–November, whereas the translucent band is already formed by March–April. The position of a typical first check for anchovy in the Bay of Biscay was also corroborated by Hernández *et al.*, (2013). Two methods were used, in the first, age was determined by identifying and measuring growth rings formed on Sagitta otoliths, and in the second, age corroboration was achieved from the otolith microstructure by daily increment counting.

In The Strait of Sicily, the back-calculation method was applied on anchovy to compare results from the growth model estimation. Back calculation of length should not be considered as neither validation nor corroboration (Campana, 2001), merely showing consistency in the interpretation.

In summary, the majority of works attempting to validate annuli of anchovy apply the qualitative method of marginal increment analysis, one of the least rigorous methods. So far, there are only two areas/stocks (Bay of Biscay and Northern Western Mediterranean) where more accurate validation methods have been used and it has been published (Morales-Nin and Pertierra, 1990; Uriarte *et al.*, 2016). There are several areas/stocks in which validations for the anchovy annual age determination have been not done yet. The provision of age validation studies should be carried out for all anchovy stocks, and especially those that are assessed analytically. Precision in age readings may be improved by workshops and otolith exchange, but the validation of the annual deposition of seasonal zones and the checks in the otolith represent the focal point to the improve the precision in the Anchovy age determination.

Table 3.2.1. Summary of age validation methods used for anchovy in Atlantic and Mediterranean areas.

Validation Method	Area	Method	Time-series	Age/Size Range	References
Marginal Increment Analysis/Edge Analysis	Bay of Biscay	Qualitative	1984–1992	Ages 0–3+	Uriarte <i>et al.</i> , 2016 (Supplementary material)
	Gulf of Cadiz		2005–2008	Ages 1–4	Millan and Tormero, 2009
	Alboran Sea		Oct. 1989–Dec. 1992	All ages together	Giraldez and Torres, 2009
	North Adriatic Sea		Jan. to Dec. 2007	All ages together/10.5–16.5 cm	Donato and La Mesa, 2009
Progression of strong year classes	Bay of Biscay	Successive modal lengths in the catches	1983–1986	Age 1–4	Uriarte and Astudillo, 1987; Uriarte, 2002
		Tracking abundance indices by age in surveys and catches	1987–2013	Age 1–3	Uriarte <i>et al.</i> , 2016
Daily increments between annuli	Bay of Biscay	Validation of first annulus	October 2012–April 2013	Age 1/8.5–13.6 cm	Aldanondo <i>et al.</i> , 2016
		Corroboration of first check	2010–2011	Age 1/11.7–20.5 cm	Hernandez <i>et al.</i> , 2013
Length frequency analysis	NW Mediterranean Sea		April 1984–Oct. 1985	Age 0–4/5–18.5 cm	Pertierra, 1987
			Jan. 1987–Jun. 1989	Age 0–4/6.5–20 cm	Morales-Nin and Pertierra, 1990

4 Validation/studies presented during the Workshop.

4.1 Bay of Biscay

During the workshop a summary of two recently published papers on the Validation of age determination for anchovy in the Bay of Biscay was presented.

a) Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay.

This a summary of the paper By A. Uriarte, I. Rico, B. Villamor, E. Duhamel, C. Dueñas, N. Aldanondo, and U. Cotano 2016. Validation of age determination using otoliths of the European anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay Marine and Fresh-water Research, 2016, 67, 951–966. The paper presented the Validation of the age determination using otoliths of European anchovy along with a historical corroboration of the method and a summary of the annual growth in length. The paper is open access and can be downloaded directly from <http://dx.doi.org/10.1071/MF15092> or from <http://www.publish.csiro.au/mf>

Validation of the age determination procedure using otoliths of European anchovy in the Bay of Biscay was achieved by monitoring very strong year classes in successive spring catches and surveys. This was first achieved with the 1982 year class which showed a neat annual progression of modal lengths passing through the fishery until the age of 4 (Uriarte and Astudillo, 1987). Validation of the proposed method was subsequently obtained through monitoring of the progression of the strong 1987, 1989, and 1991 year classes, both by spring annual surveys and by continuous sampling of the commercial catches, coupled to the monitoring of the seasonal marginal edge formation of the otoliths.

Since then, historical corroboration of the ageing method was obtained by the statistically significant cross-correlation between successive age groups by year classes in catches and surveys (1987–2013).

Typically, annual growth of anchovy otoliths of the one and two years old diminishes to about a half and a third of that occurring in their previous ages respectively. Otolith growth during the first year of life (as age-0, until first winter hyaline zone) is vast and usually supposes the major part of the otoliths (even the oldest ones). During the second year of life (age-1), the opaque growth between first and second winter hyaline zones is still substantial though reduced to about 50% (CV = 44%) compared to the growth achieved at age-0. And during the third year of life (age-2) the reduction of the opaque growth zone is very much pronounced, to about 29% (CV = 33%) of that achieved at age-1. In subsequent ages, opaque growth still diminishes but to a lesser extent than the trend shown during the first three years of growth. As such, at age-3 opaque growth is still about 48% (CV = 34%) of that produced at age-2, and at age-4 growth is probably larger than 50% of that achieved at the age of 3 as deduced from the few ages 5 of the 1982 cohort. In summary, the decreasing rate of annual growth with age (relative to the former age) is not constant but accelerates from ages 1 to 2 then slows down from age-3 onwards, leading to a gradual (less intense) narrowing of increments subsequently. This typical pattern of otolith growth is clearly shown by the oldest ages Figure 1 of the paper presents typical pictures of otoliths from ages 1 to 5 in Spring, both without or with false rings (or checks). In addition, the first section of the electronic supplementary material of that paper contain another set of picture of Otoliths of European anchovy in the Bay of Biscay throughout the year along with a seasonal characterization by age classes, as seen by incident light on whole mounted

otoliths over black slides. In those Figures, the typical growth pattern is clearly observed. Additional examples of typical otoliths at age 0 (autumn) and at ages 1 to 4 in spring for a recent cohort are shown here in Figure 4.1.1 (which were not included in the original published paper).

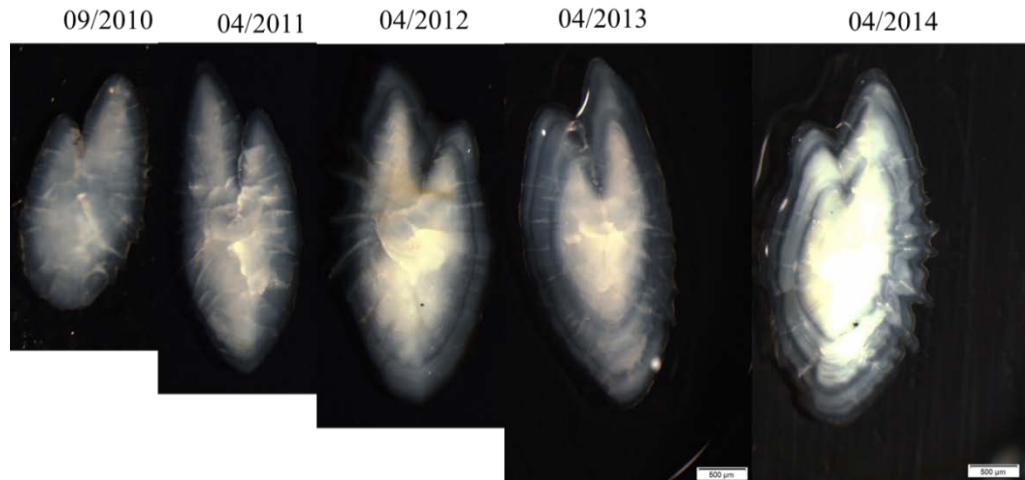


Figure 4.1.1: Typical Otoliths at age 0 (Autumn) and at ages 1 to 4 (Spring) corresponding to the 2010 year class (Source AZTI; in Uriarte *et al.* 2014 presentation).

Most opaque growth occurs in summer and is minimal (translucent) in winter. Effectively, maximum occurrence of opaque growth edges occurs with the warming up of temperatures in late spring and summertime, while maximum occurrence of hyaline zones for all ages corresponds to the period of coldest seawaters in late winter. This seasonal formation of the margin edge differs remarkably between ages, with the young anchovy (the 1-year-old) resuming opaque growth earlier (since February or March) than the older (at late spring or early summer -- May or June) (Figure 4.1.2). This feature helps distinguishing age-1 from age- 2+ during the first half of the year. The actual formation of the otolith edge throughout the year by age classes is described in detail in the paper and the electronic supplementary material (Uriarte *et al.*, 2016).

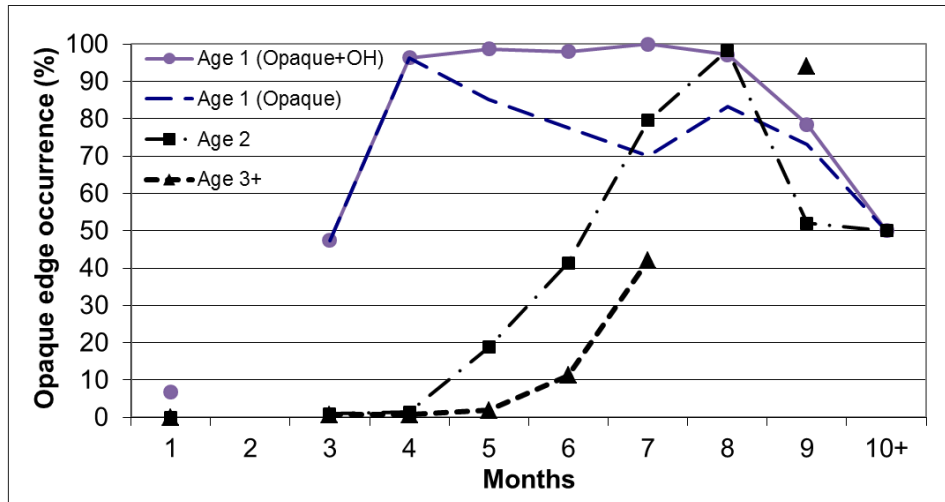


Figure 4.1.2: Occurrence of marginal opaque edges (adding up the opaque narrow and wide edges: ON + OW) by age class and month for the Bay of Biscay anchovy. Age-1 is shown either including the new semi-hyaline edges (opaque edges + OH2) which occur during summertime or excluding them (Opaque = ON+OW). New semi-hyaline edges (OH2) refer to the transition forms from opaque to hyaline not entirely visible all around the margin of the otolith which appear after having resumed (or completed) the annual marginal opaque growth (Taken from Uriarte 2016 Phd). This graph allows concluding that the younger the fish the sooner resumes opaque growth in the year; in spring edge of age 1 is typically opaque, while it is hyaline for older ages.

During the first winter several translucent rings are occasionally formed resulting in a composite annulus formation. In addition during June/July, at peak spawning, a check is formed in many of the one year old anchovies (see pictures in Figure 1 of the original paper). However, not all year classes, neither all anchovies lay down the same amount of checks and many of them may not show any. The differences between true winter annual ring and the checks can be difficult: Usually checks tend to be weaker or more diffuse than true annual rings and often they are not completely formed all otolith around.

Therefore, Yearly annuli consist of a hyaline zone (either single or composite) and a wide opaque zone, disrupted occasionally by some typical checks (mainly at age-0 and at age-1, the latter usually formed at peak spawning time).

Age determination, given a date of capture, requires knowledge of the typical annual growth pattern of otoliths, their seasonal edge formation by ages and the most typical checks.

In summary, for this anchovy with its birthdate at 1 January, the two ageing criteria for anchovy in the Bay of Biscay, agreed in the 2002 workshop (Uriarte *et al.*, 2002; ICES 2009 and in Uriarte *et al.*, 2016) are:

- 1) Criteria of complete annual growth zones contained in the otolith (annulus) in conformity with the typical annual growth pattern of this anchovy, so that assigned age equals the number of complete opaque growth zones corresponding to the expected annual growth pattern of the otoliths, excluding the marginal edge development of the year. The latter arise from the fact that hyaline zones are usually formed in winter during the first months of the year and are not necessarily present since the beginning of the year. Typically successive annual opaque growth zones are expected to be of decreasing width (Morales-Nin and Panfili, 2002), but the particular decreasing growth pattern of this anchovy is the first general scheme age-readers

should be aware of for the examination of these otoliths (as described above and in Uriarte *et al.*, 2016). In case the opaque zones do not correspond to the typical expected annual growth pattern for the presumed age, the existence of some false rings or checks can be suspected and evaluated.

- 2) The criteria of conformity of the marginal edge development with the expected type of edge at the month (season) of capture which does change by ages (particularly for age-1). Most opaque growth occurs in summer and is minimal (translucent) in winter, but taking into account that the opaque edge formation begins sooner at the age of 1 (around February-March) than at older ages (May or June). Age readers should know this dynamic pattern of the edge to assess conformity with the current criteria. If the edge of the otolith does not correspond to the expected edge type of the assigned age at the date of capture (as deduced from the former criteria), then alternative interpretations could be considered. Given that age-1 is the usual predominant age class in the anchovy population this is a relevant criteria to differentiate between ages 1 and older, particularly during the first half of the year.

Finally, for the correct interpretation of otoliths according to these criteria the reader should also be aware of the typical checks as explained above.

b) Validation of the first annual increment deposition in the otoliths of European anchovy in the Bay of Biscay based on otolith microstructure analysis.

This is a summary of the paper by Naroa Aldanondo, Unai Cotano, Paula Alvarez, and Andrés Uriarte (2016). Validation of the first annual increment deposition in the otoliths of European anchovy in the Bay of Biscay based on otolith microstructure analysis *Marine and Freshwater Research*, 2016, 67, 943–950. This paper validates the first annual increment deposition through the study of the daily growth of otoliths of reared juvenile anchovies. It is an open access paper and it can be downloaded from <http://dx.doi.org/10.1071/MF15083> or from <http://www.publish.csiro.au/mf>.

In order to validate the first annual increment deposition in European anchovy otoliths, early juveniles were captured in October 2012 in the southern Bay of Biscay. These individuals were maintained under a continuous feeding regimen in a sea cage over a period of 6 months. From October 2012 to January 2013, lengths increased slightly or remained stable at around 9.8 cm. After this period, standard length increased significantly up to a mean value of 12.0 cm in April 2013. Likewise, the age of anchovies was estimated based on otolith microstructure analysis. The estimated age varied from 96 days (for individuals sampled in October 2012) to 293 days (for anchovies sampled in April 2013). A daily increment deposition rate was confirmed in otoliths of individuals maintained in the sea cage during winter (Figure 3 of the paper proved such a relationship).

The general otolith daily growth pattern showed that increment widths increased rapidly and were broadest between 51 and 56 days, with a mean of 19.1 μm . Thereafter, the widths decreased steadily to 1.5 μm and remained almost constant until the end of the experiment. Figure 4.1.3 shows the typical daily growth increments according to the age of the anchovy juveniles throughout their first year of life and the general parallelism with the changes in temperature.

The present study also revealed that the first translucent band formation started in autumn and was completed by spring. This translucent band was characterized by a decline in increment widths, which were significantly narrower than those in the adjacent

opaque band (Figure 4.1.3). Examples of entire and polished otoliths showing the inner opaque area and the more translucent outer region appear in Figure 4.1.4 below.

This general pattern of growth of the otoliths observed just after exceeding their first winter period was observed in many anchovies from all studied areas in the workshop: Figure 4.1.5 presents some examples of this general pattern as observed in entire otoliths with transmitted light imbedded in resin, where the opaque inner zone is shown whiter than the outer translucent zone which is seen just as a more grey zone (less whiter than the other), just disrupted by one or more thin complete hyaline rings.

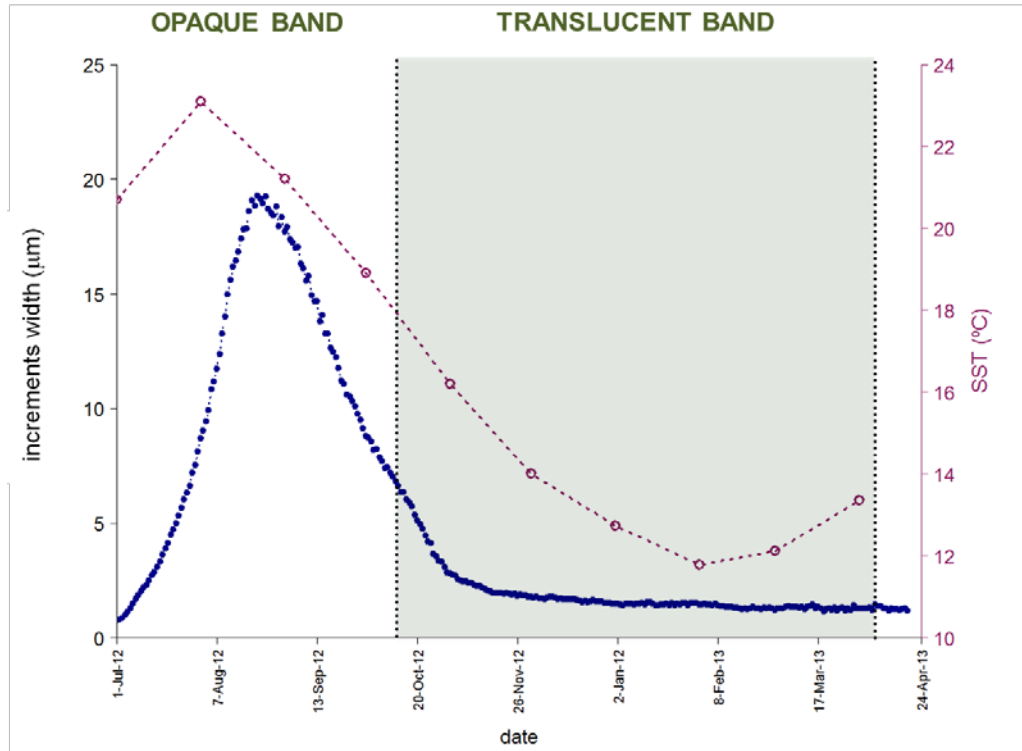


Figure 4.1.3 Average increment widths at age from otoliths of anchovy juveniles (blue dots) and the monthly mean sea surface temperature (SST) values obtained from data provided by the Aquarium of San Sebastian (438190N, 028W) (discontinuous line with circles). The shadow area, bounded by the vertical dashed lines, corresponds to the period of translucent band formation in the otoliths. (Source: Merging Figures 6 and 7 of the Aldanondo *et al.*, 2016 paper.

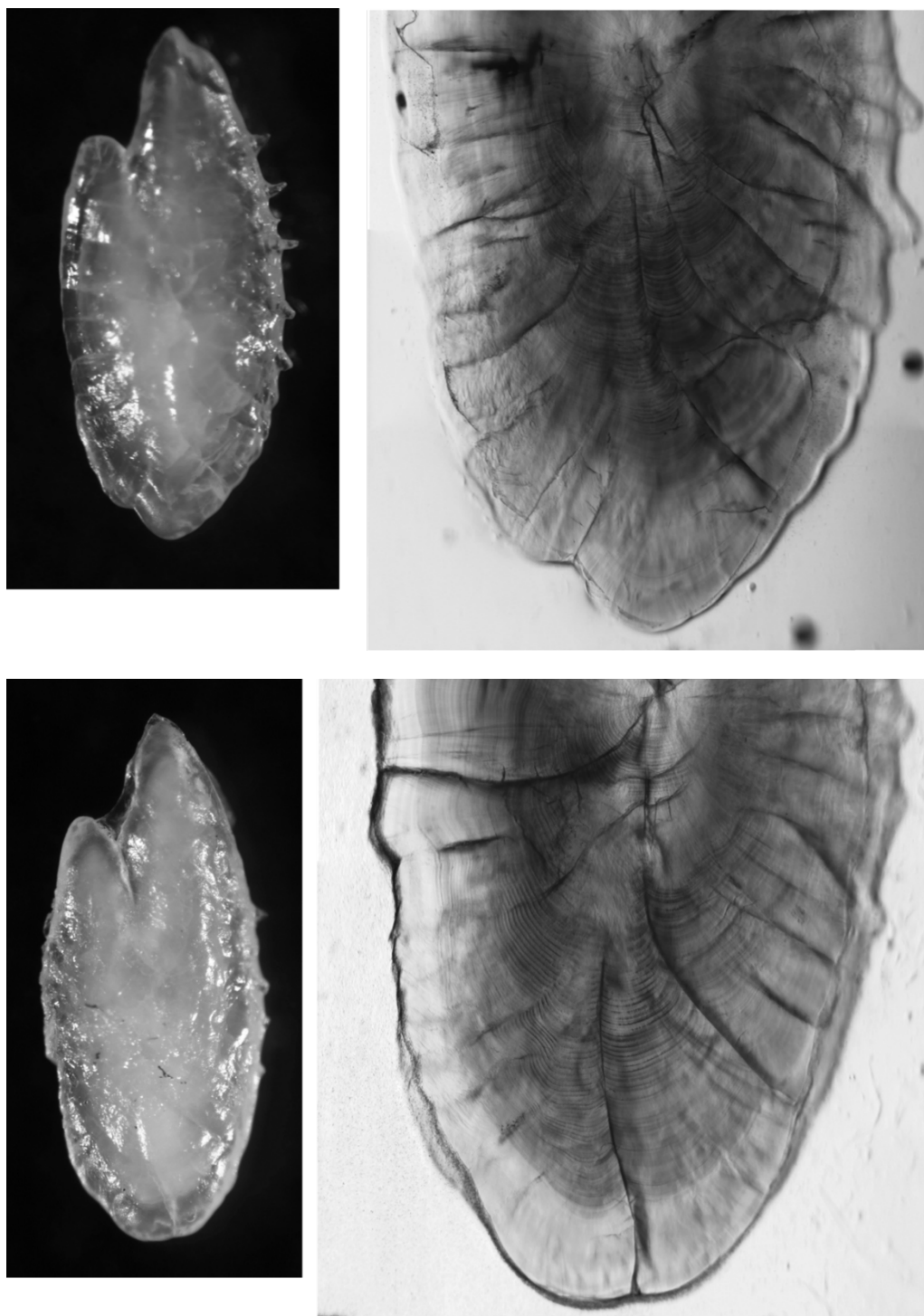


Figure 4.1.4: Pictures of two entire otoliths (with incident light) at the end of the reared experiments, examined by Aldanondo *et al.* (2016) (upper and bottom left figures) and corresponding polished posterior part of the otoliths (with transmitted light) where the outer more translucent area can be seen (upper and bottom right figures) (Source: Aldanondo pers. comm.).

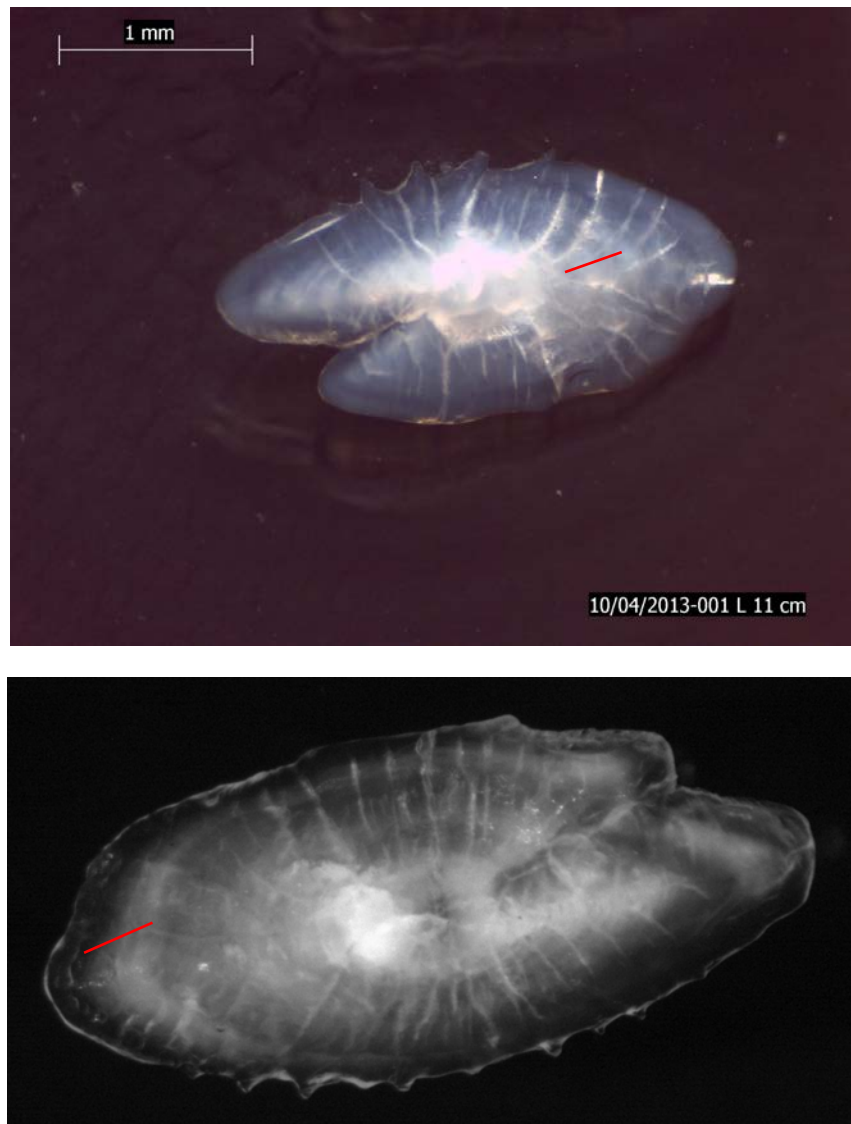


Figure 4.1.5: Upper picture: an otolith of region GSA 06 caught in April 2013 (Fish length 11 cm), probably a 1-year old with a growing opaque edge, where the first winter (October-February) period is marked by a red line. Bottom picture: an otolith of region GSA 22 (Ee_109_03_EVOIKOS_28-4-14_L_25) taken in May 2014 (Fish length 10.9 cm), probably a 2-years old with a relevant margin opaque growth, where the first winter (October-February) period is marked by a red line (Sources: IEO / ELGO respectively).

4.2 Division 9a

4.2.1 Subdivision 9a North (Southern of Galician waters)

This is a summary of the presentation made to WKARA2 on the Criteria for age determination of anchovy otoliths in Subdivision 9a North: analysis of the rings biometric measures, by Hernandez, C., Dueñas-Liaño, C., Antolinez, A. and Villamor, B. Instituto Español de Oceanografía. C.O. de Santander. Spain.

The fishery is occasional with phenomena of sporadic and high increases in the availability of anchovy in the 9a-N region (area covering the coasts of western Galicia and northern Portugal). Probably the increased availability of anchovy in the 9a-N region is due to an exceptional increase in local residual populations. But it could also be that the increase in the availability of anchovy in the 9a-N region is a consequence of the

increase of shoals coming from one of the established populations (Division 9a or Sub-area 8).

Catches from this Subdivision only accounted for about 2% of total catches in the whole Division 9a. Size range in catches from the whole fishery was comprised between 11.5 and 17.0 cm size classes (mode at 14.0 cm size class), with an annual mean size and weight in catches being estimated at 14.5 cm and 21.0 g, respectively. The catch of anchovy from the IEO annual acoustic survey (PELACUS, March-April) is very scarce. (ICES WGHANSA, 2016)

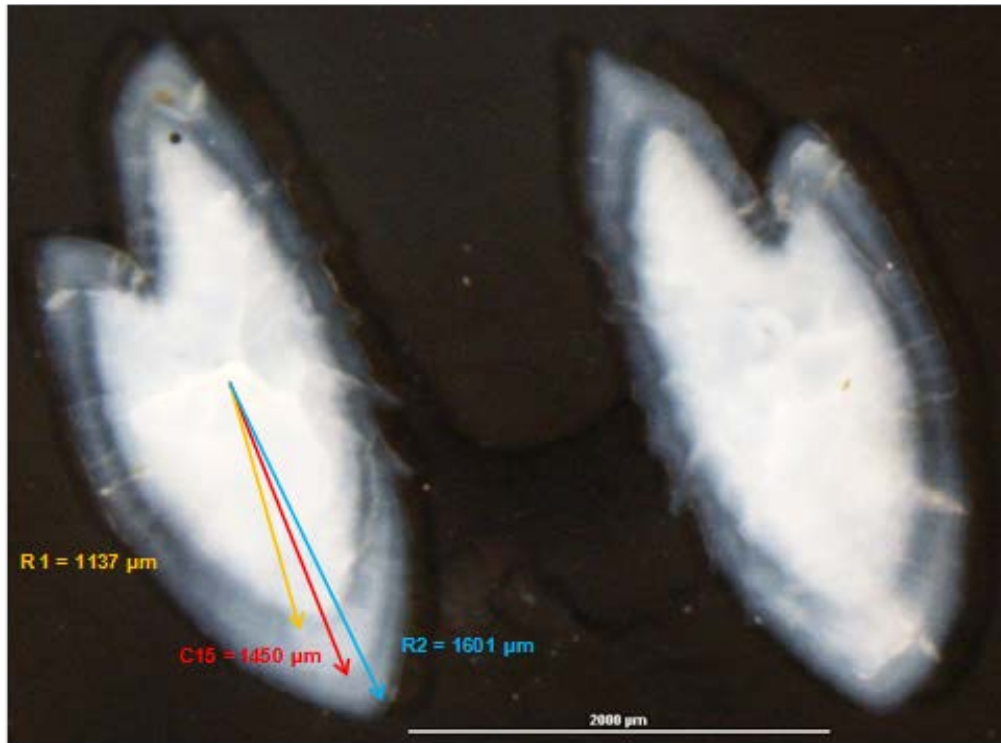
Biological sampling and otoliths reading only carried out in the years of higher catches. Typical Length range: 11–17 cm; Age range: 1–3 years. Otoliths of this area have never been included in the exchanges or workshops.

Two methods were used to study the growth pattern of the anchovy otoliths in the Subdivision 9a North: 1) Age was determined by identifying and measuring growth rings formed on the *sagitta* otoliths in Subdivision 9a North, in order to support the identification of the true annual rings: The otolith radius of the hyaline rings was measured and used as a gauge to exclude the presumed checks in ageing older individuals. 2) Also, the nature of the edge (hyaline or opaque) was also recorded.

Analysis of whole otoliths: rings biometric measures

A total of 141 otoliths of *Engraulis encrasicolus*, in the total length (TL) range 10.5–17 cm from commercial sampling in 2016 were analysed under a reflected light, using a microscope applied to an image analyser (NIS-Element). Macroscopically visible rings distances were measured (C08, C15, R1, R2) along the same axis used to estimate the age of the fish. 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 checks and annual growth rings distances were measured between the core and the inner edge of hyaline rings (Figure 4.2.1.1), 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 following the age determination criterion of the Anchovy Bay of Biscay (ICES, 2009; Uriarte *et al.*, 2016).

Examples of measures: Age 2 with a check C15



22/06/2016. N° 12, 130mm. Check C15. Hyaline. Age 2

Figure 4.2.1.1 Measurements taken from anchovy otoliths. Presumed check = C15, R1 = first annual hyaline ring, R2 = second annual hyaline ring

The results showed that increments widths have a normal distribution (Kolmogorov–Smirnov test, Presumed Checks, R1, R2, and R3 values $p > 0.05$) with a falling rate of otolith growth with age (Figure 4.2.1.2). This linearly decreasing interval between increments is a verification criterion that forms the basis of age estimation (May, 1965).

In cases where the distance from the core to the first visible ring was $< 779 \pm 95 \mu\text{m}$, this ring could be assigned by the reader as the presumed check C08. Based on the measurements made, a guideline table for age interpretation was established (Table 4.2.1.1).

Table 4.2.1.1. Guidelines for age estimation. The distance between the core to the beginning of the presumed checks and winter rings (μm): average, standard deviation (s.d.), and minimum and maximum values observed.

Rings	Average (μm)	s.d	Minimum (μm)	Maximum (μm)
C08	779	95	573	946
R1	1213	186	928	1715
C15	1370	136	1125	1671
R2	1643	147	1341	1908

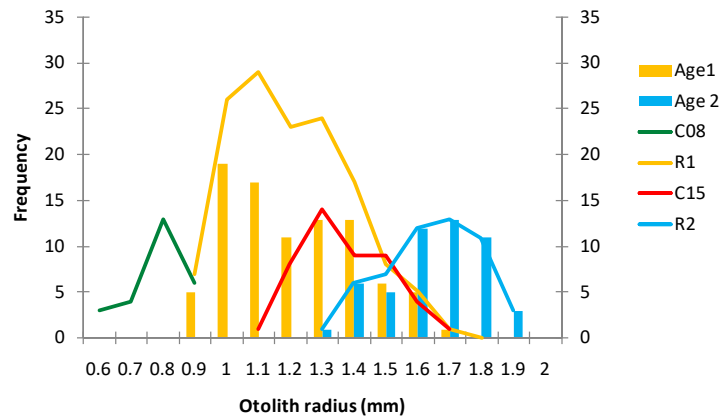


Figure 4.2.1.2. Frequency distribution of rings distances. Presumed checks, R1, R2, R3 and tree annual age ring distances.

Nature of the edge

1122 whole otoliths from commercial sampling in 2015 and 2016 were analysed. The nature of the edge (hyaline or opaque) was recorded.

The monthly proportion of edge type of *E. encrasicolus* in the 9aN, indicates an annual periodicity in the formation of the hyaline and opaque annuli, appearing the hyaline edge mainly from June to October. (Figure 4.2.1.3)

At age 1, the highest frequency of occurrence of opaque edge occurs in winter, descending in summer. This could be assumed as a stop on growth at age one, in summer. At age 2, the highest frequency of occurrence of the opaque ring occurs in summer (Figure 4.2.1.4). These results, contrary to expectations (hyaline in fast growth season, opaque in slow growth season), could be due: presumably due to the formation of spawning checks or also, it could be due to a greater influence of the upwelling in that area (Cabanias *et al.*, 1999), mainly in summer, with consequent decrease of the temperature, which could cause the expected pattern of edge formation to be altered.

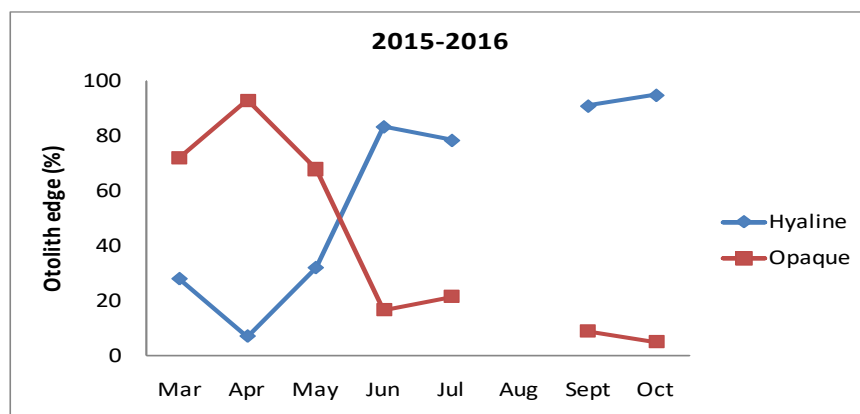


Figure 4.2.1.3. Monthly proportion of edge type in Anchovy of Subdivision 9a North (2015–2016 combined).

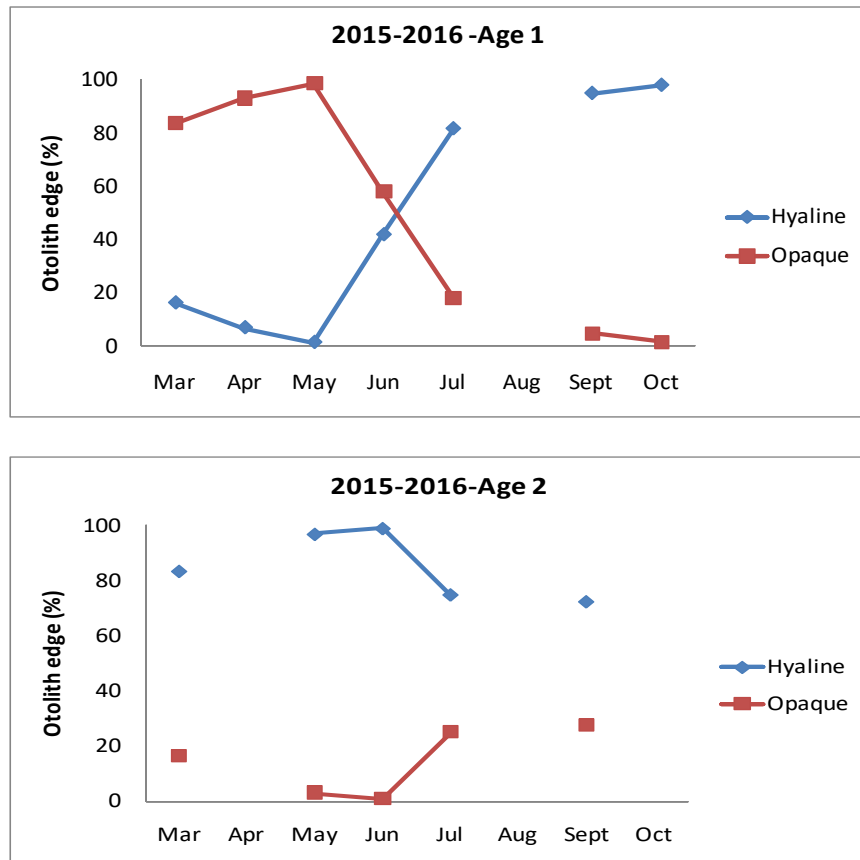


Figure 4.2.1.4. Monthly proportion of edge type in Anchovy of Subdivision 9a North (2015–2016 combined) by age.

Conclusions

The biometric measurements of the growth rings (true and false) of the anchovy otoliths in the North 9a ($C08 = 779 \mu\text{m}$, $R1 = 1213 \mu\text{m}$, $R2 = 1643$), appear to be in agreement with the results obtained for the anchovy otoliths from the Bay of Biscay ($C08 = 852 \mu\text{m}$, $R1 = 1295 \mu\text{m}$, $R2 = 1589$, Hernandez *et al.*, 2013).

It is necessary to investigate more in depth the analysis of the nature of the edge, since it seems to indicate that the growth is greater in winter than in summer. Could this be explained by the temperature drop due to upwelling phenomena, in this area in summer, and therefore would cause a decrease of anchovy growth or is it simply due to the formation of spawning checks.

4.2.2 Subdivision 9a South (Gulf of Cadiz)

Two studies in preliminary stage were conducted for the workshop with data from ICES 9a-South otoliths. This work is the summary of the presentations made in WKARA2 (Presentation 4): Tornero, J. Gulf of Cadiz anchovy otoliths: A preliminary study on the evolution of radius of A1 ring and post-rostrum radius through the year and evolution of the edge through the season. IEO.

Evolution of radius of A1 ring and post-rostrum radius through the year. From a total of 892 otoliths from 2009 samples, only 342 were selected due to the high confidence in correct A1 assignment. Then, radius to A1 (Ra1) and radius to post-rostrum (Rpr) edge were measured, and then other measurements' were derived:

- Season's growth (Sgr), as the difference between Ra1 and Rpr
- Ratio between Sgr and Ra1
- Ratio between Sgr and Rpr (RPS)

Basic statistical parameters were calculated on those magnitudes as depicted in the table below. The mean distance from nucleus to A1 ring was 1237.27 μm , close to the mean distance on other study areas (Figure 4.2.2.1 and Table 4.2.2.1).

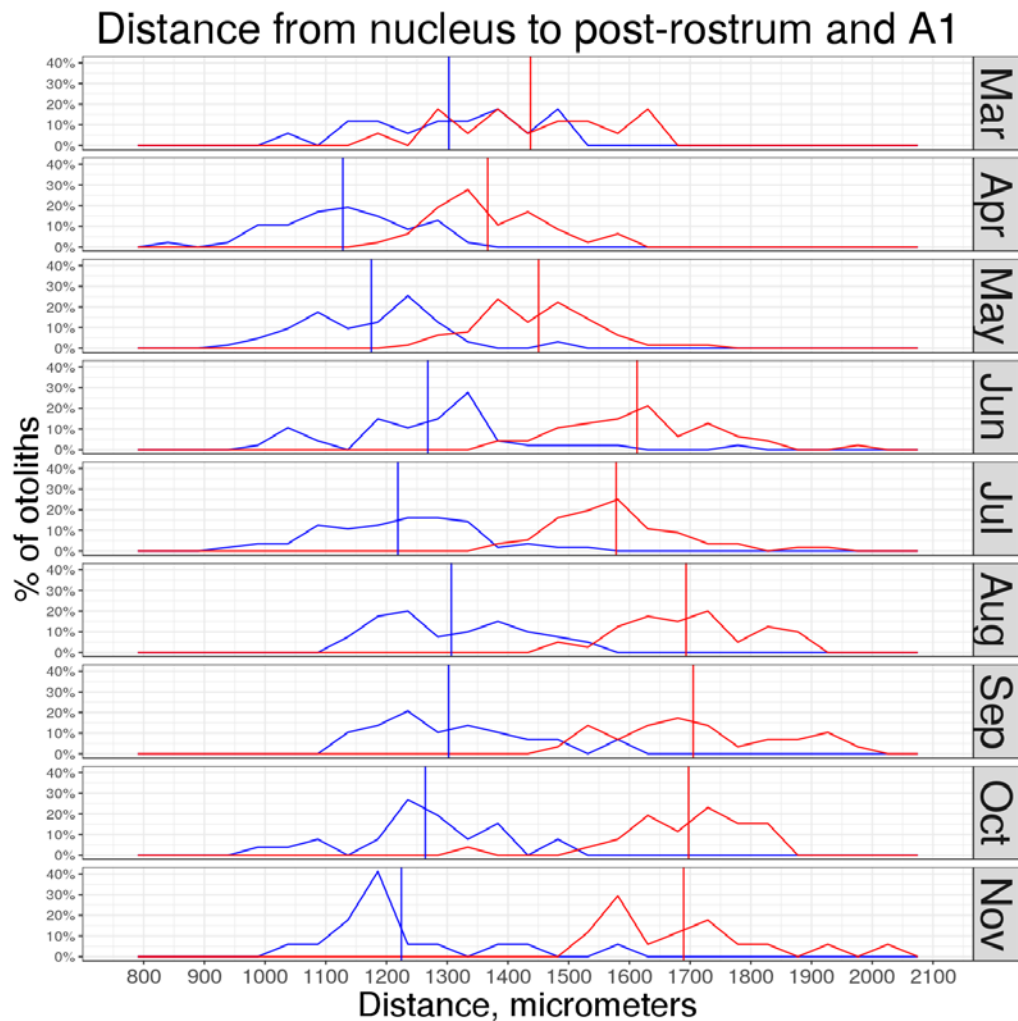


Figure 4.2.2.1. Measurement of the distances from nucleus to post-rostrum (red lines) and to the first winter ring (A1) (blue lines) by month

Despite the evolution of these magnitudes through the year seem to follow a pattern; it should be further investigated due to the preliminary status of the study.

Table 4.2.2.1. Mean distance between the core to the postrostrum (Rpr) and to the first winter ring (RA1)(μm) and standard deviation (s.d.), along with minimum and maximum values observed.

	TL (mm)	Rpr (μm)	RA1 (μm)	Sgr (μm)	Rpr
Min	93	1180	861	45	0.04
Max	162	2021	1790	820	0.43
Mean	124.02	1562.14	1237.27	331.87	0.21
SD	14.56	166.37	136.62	114.22	0.06

Also, a study of the edge evolution (opacity) of the otoliths was conducted. In this case, both percentage of hyaline edges vs. total number of otoliths and opaque edges vs. total number of otoliths were calculated from otolith edge data from 9842 otoliths collected during years 2012 to 2015. Data were aggregated by month and plotted by age and all age groups together (Figure 4.2.2.2).

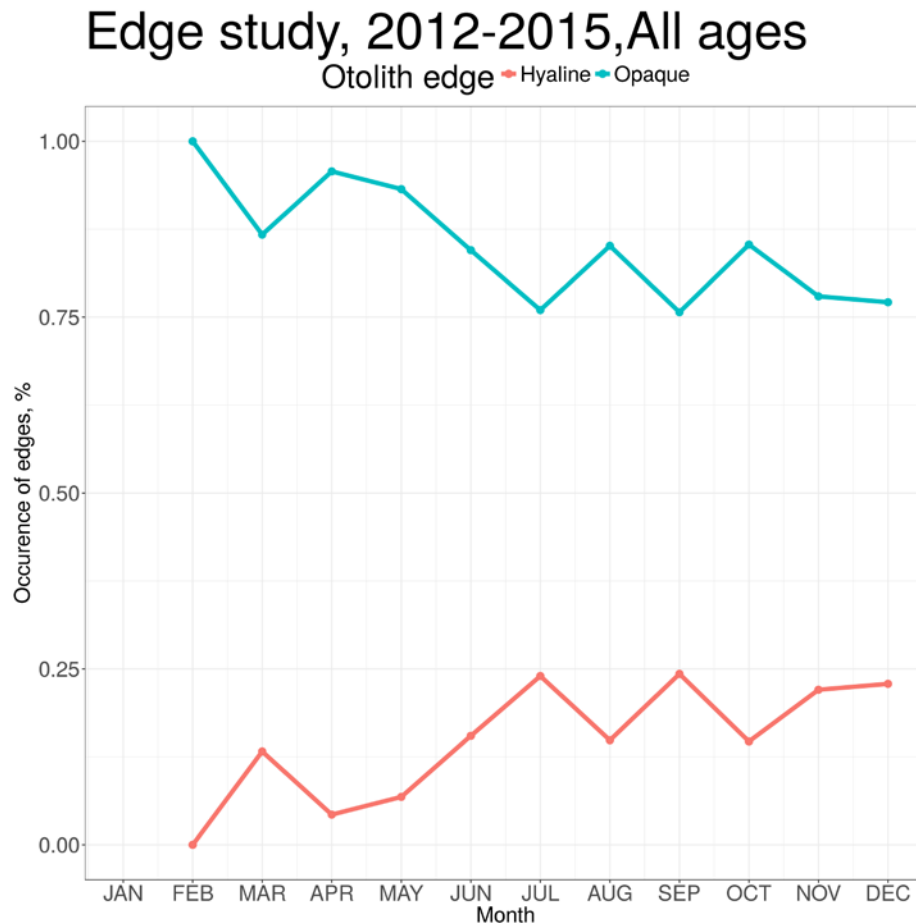


Figure 4.2.2.2. Monthly proportion of edge type in Anchovy of Subdivision 9a South (2012–2015 combined)

Despite the lack of observations in some months (November–December to January–February) due to fisheries regulations, some interesting features could be observed. For age group 0 (which occurs only in the second semester due to age assignment restrictions), must be mentioned that both hyaline edges in late summer (before what should be expected at first) and opaque edges in early winter could be noticed (Figure 4.2.2.3).

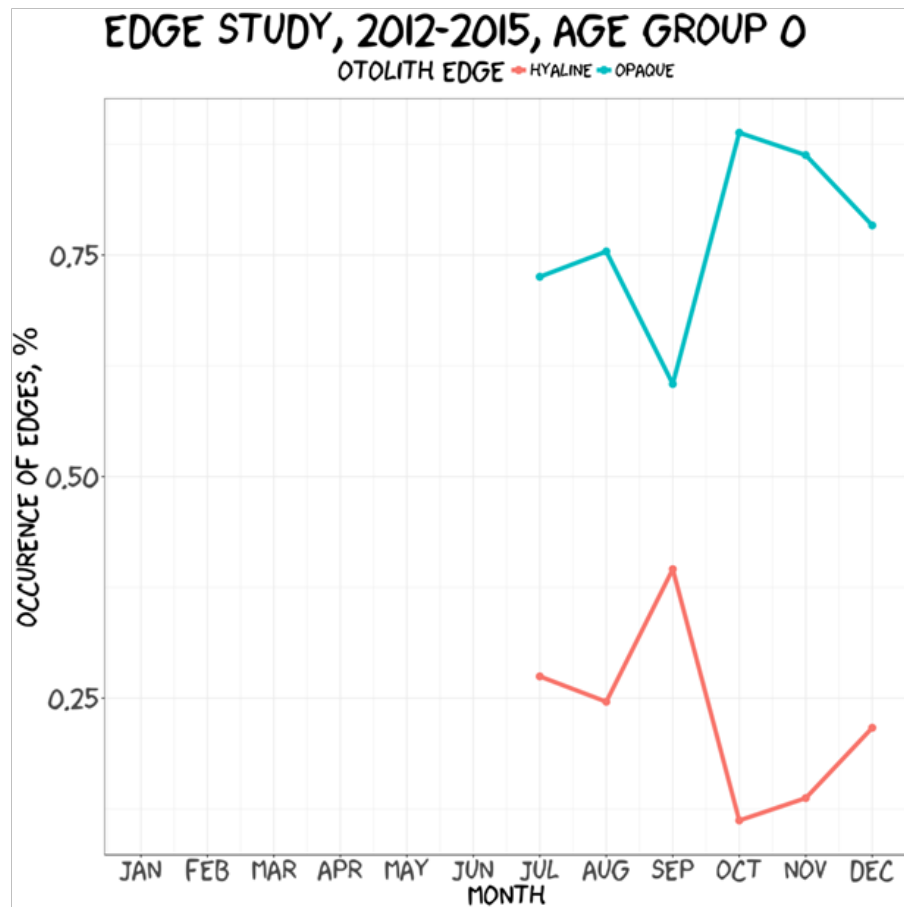


Figure 4.2.2.3. Monthly proportion of edge type in Anchovy of Subdivision 9a South (2012–2015 for fish of age group 0).

For age group 1, the most noticeable fact is the presence of a relatively high percentage of hyaline edges during summer, presumably due to the formation of spawning checks (Figure 4.2.2.4).

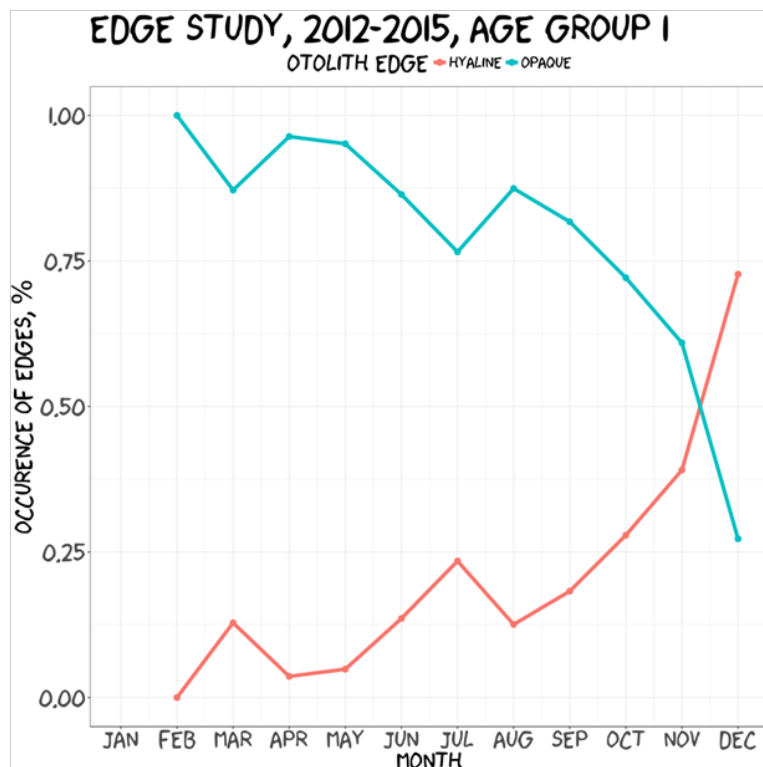


Figure 4.2.2.4. Monthly proportion of edge type in Anchovy of Subdivision 9a South (2012–2015 for age group 1).

The lack of observations of age group 2, samples produced very noisy plot for that age and preventing obtaining any conclusion from such information (Figure 4.2.2.5).



Figure 4.2.2.5. Monthly proportion of edge type in Anchovy of Subdivision 9a South (2012–2015 combined).

In any case, the relatively high abundance of *unexpected* (hyaline in fast growth season, opaque in slow growth season) otolith edges could favour wrong age-group assignments.

4.3 Alboran Sea

This section is a summary of the Presentation 5 to this workshop: Torres, P. (IEO) Alboran Sea and Western Mediterranean anchovy: Fishery data (catches, lengths, weights, and evolution of modal length) and studies of otoliths (microstructure and evolution of edge).

Microstructure

An exercise for microstructure was done previously to the WKARA2 using a new software at present in development in our institution OTOLAB (Figure 4.3.1, Nava *et al.*, in press). The purpose of OTOLAB is helping marine scientists in their research activities on otolith structure to estimate the age of black hake individuals. The software is also helpful with otoliths of other species. In future, it is intended to be used also with bivalvia (e.g. clams) and mollusca (e.g. octopus). OTOLAB is considered to be Open Source Software since the code is freely available so that every user can modify or improve its performances at his convenience. On the other hand, OTOLAB is a MATLAB script developed with the help of MATLAB GUIDE and, as a consequence, MATLAB Software, which is not Open Software, is needed to run it.

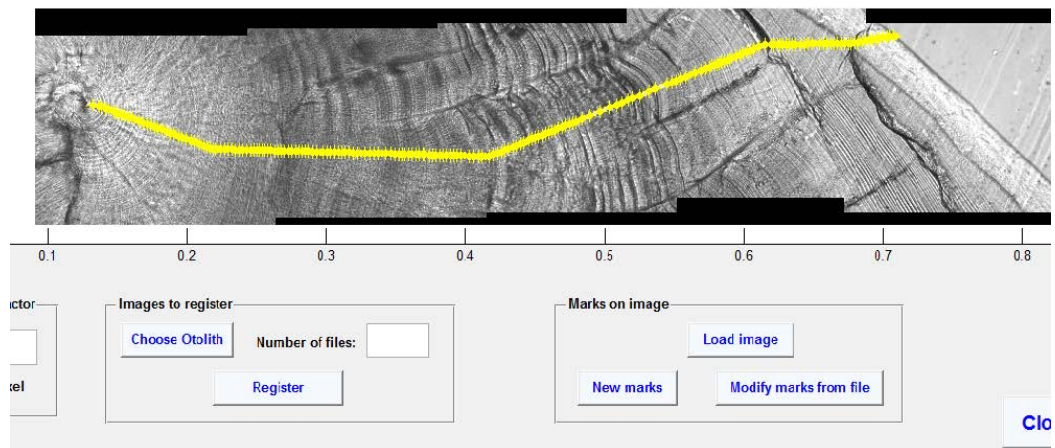


Figure 4.3.1. Microstructure otolith image slice: A new software at present in development in our institution OTOLAB.

We prepared just a few otoliths as this was only a test using the software with anchovy's otoliths. The results were promising and shown an encouraging way to work with microstructure. This technique has been used satisfactorily in black hake (*Merluccius* spp.) until two years old.

In our study case for anchovy of 15 cm total length were counted around 200 daily increments, showing a very fast growth although they were caught in March (winter is supposed to be the slow growth season) (Figure 4.3.2).

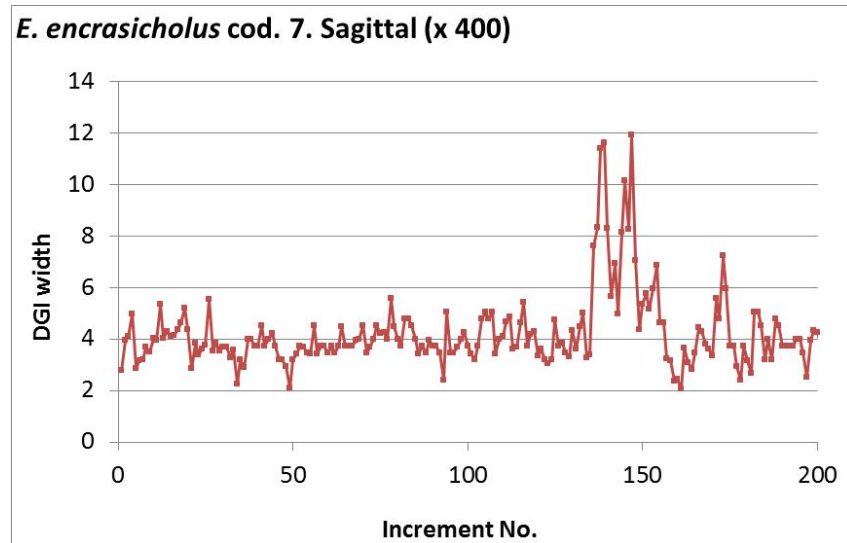


Figure 4.3.2. Graph showing the number of daily increments and their width.

Following cohorts through time

Doing data mining to prepare the WKARA2 we found very interesting information to show the growth of cohorts through time. Until 1998 the fishery was basically focused on the recruitment at the end of the year (mainly autumn) (Figure 4.3.3).

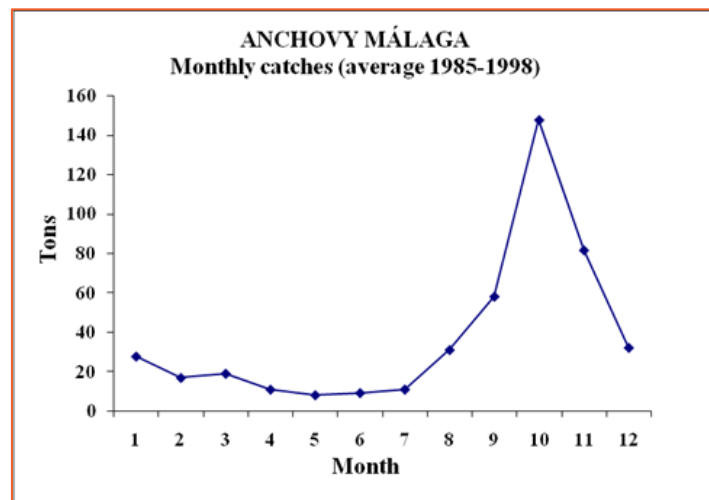


Figure 5.3.3. Mean monthly landing from 1985 to 1998, showing the peak at the end of the year.

Modal length for each length distribution and month were calculated for the period 1989–1991 when recruitment was caught showing the growth of the cohort, in 1992 the recruitment was not caught. As we may infer from the graph growing from size 5 to 7 in autumn to size 13–14 the next spring-early summer. (Figure 4.3.4)

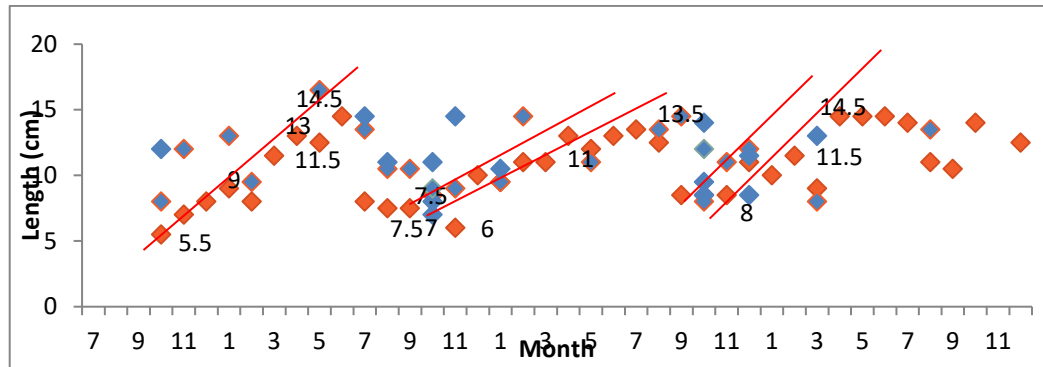


Figure 4.3.4. The growth of the anchovy cohorts from 1989 to 1991.

Radius to the 1st annulus

The radius to the 1st hyaline ring (2014) were measured, taking into account only level 1 of the quality grading system (WKNARC 2011, level 1 = easy to age with high precision, it is an indication that the age data are considered reliable for stock assessment). The results were as follow in Table 4.3.1.

Table 4.3.1. Values for the radius of the first true winter ring in GSA01 and 06 for 2014 taking into account only level 1 of the quality grading system (WKNARC 2011).

Units (mm)	R1 GSA01	R1 GSA06
Mean	1.405	1.301
Desvest	0.147	0.137
Min	1.1	1.05
Max	1.8	1.73
n	92	74

Such values are quite similar to the radius for the first annulus in anchovy as it was recommended in WKMIAS 2013. In the case of Alboran Sea-GSA01 anchovy shows a bigger size at age than in Northern Mediterranean coasts of Spain-GSA06.

Monitoring of the hyaline edge formation (shown in WKARA 09)

This study determined the seasonality in the formation of rings in the anchovy otoliths by monitoring hyaline edge in the Alborán Sea. Monthly samples were collected between October 1989 and December 1992 from the catches of the purse-seine fleet. The formation of hyaline rings is significantly correlated with the surface temperature of seawater. The highest percentage of hyaline edge formation, recorded over the four year study, periodically occurred in February, when the temperature is at its lowest point. The monthly percentage of hyaline edge is shown in Figure 4.3.5. The highest percentage of hyaline edge occurs between December and February, with a maximum in February every single year. The minimum occurred between August and November. There are never a 100% of individuals forming a hyaline ring.

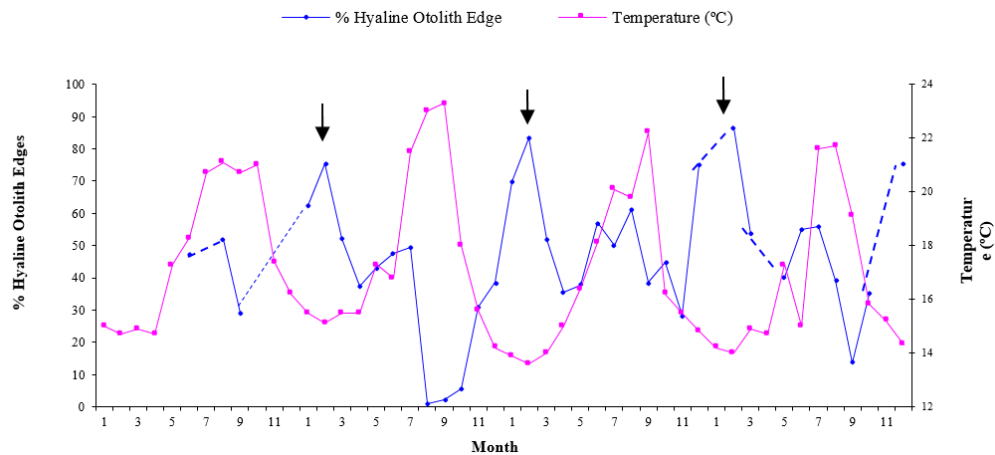


Figure 4.3.5. Monthly evolution of percentage of hyaline edge formation and seawater temperature (dashed line without data).

4.4 Western Mediterranean

Since the validation studies of Morales-Nin and Perterra (1990) (based on length frequency studies), no new validation/studies were carried out for this area.

4.5 Gulf of Lion

No validation/studies were carried out for this area.

4.6 Tyrrhenian Sea

No validation/studies were carried out for this area.

4.7 Strait of Sicily

This is a summary of the presentation No.6 of Gualtiero Basilone, Salvatore Mangano and Maurizio Pulizzi (IAMC-CNR). Preliminary results on daily growth increment pattern in European anchovy (*Engraulis encrasicolus*) juvenile otoliths in the Strait of Sicily (GSA 16).

In the Central Mediterranean (GSA 16), age and growth data on juveniles were obtained by otolith daily increment analysis of European anchovies collected in the 2005. Daily otolith age readings permitted to evaluate the birthdate distributions, which displayed a peak between July and August with ages ranging from 52 to 325 days. Variability of the growth pattern across the seasons was analysed and anchovies belonging to different birthdates provided a general trend of the growth pattern. An initial slow growing period during winter seems disappear in fish born at the end of winter or in spring, when starts a fast growing period in summer, representing by a thicker opaque band. In individuals born late in summer or early in autumn is visible a first hyaline zone followed by a wide opaque laid down during the spawning period, which contain one or two different checks (spawning checks). A clear evolution across the year of the mean increment width, of the spring-summer growth, which increases gradually according to the distance from the peak of spawning time of anchovy. The evaluation of oceanographic parameters and growth performances showed a similar trend along the year between sea surface temperature and the increment width, suggesting better growing performance with warmer, summer waters.

Sampling of anchovy juvenile

Juvenile samples of European anchovy were collected during the recruitment surveys conducted on board the RV “*Dallaporta*” on 5–13 October 2005. The catches were performed by means of a midwater trawlnet with an opening of 14 x 7 m, and with the codend mesh-size of 19 mm (stretched) in the Strait of Sicily (Figure 4.5.1).

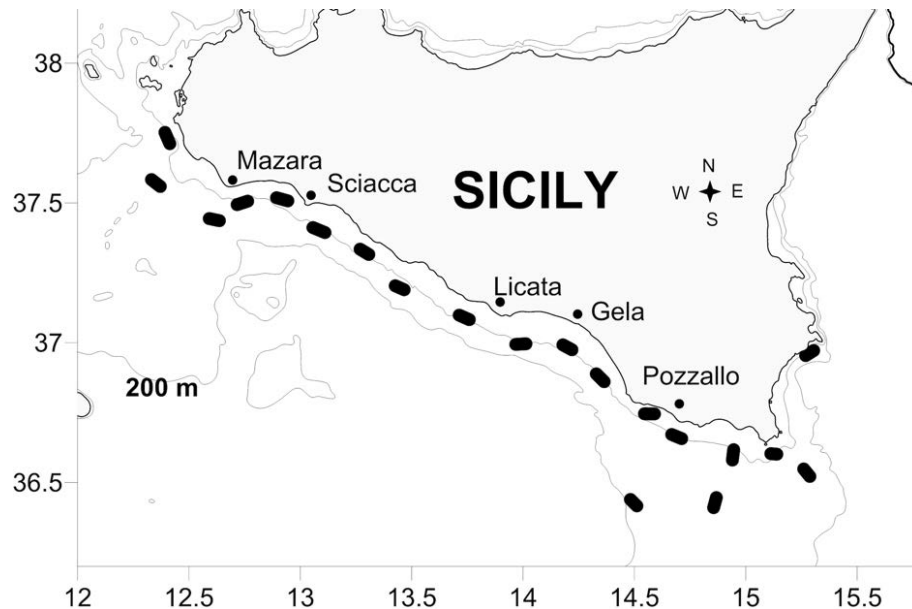


Figure 4.5.1. Map of the Strait of Sicily with geographical positions of performed trawls (black dashes).

Among the performed trawls (20) only 9 were positive to anchovy. For each positive trawl an anchovy random subsample was measured on board in order to obtain total (TL) and standard length (SL) to the nearest 1 mm, and total body weight (TW) to the nearest 0.1 g by means of a scientific portable marine scale (*Marel S182*). Otoliths were extracted from specimens up to 130 mm TL, i.e. the mean measurement which represents the length when anchovy juveniles are expected to become adults (one year-old juveniles; Basilone *et al.*, 2004). For each trawl, 25–50 sagittal otolith pairs – five specimens for each 0.5 cm size class – were extracted, cleaned and dry-stored in black plastic moulds. The trawl codend mesh-size did not allow retaining specimens less than 45 mm SL.

Otolith preparation and readings

The otolith preparation for micro-increment and birthdate analyses were performed on otolith from specimens to cover the complete size range of juvenile size distribution (Basilone *et al.*, 2004). A total amount of 310 otoliths, one for each specimen, were collected for polishing; each otolith was fixed on a microscope glass slide by means of resin (*Entellan®Merck*), and thin sagittal sections were produced by grinding with decreasing grain paper. The same procedure was adopted on the opposite side to obtain a very thin section, which was then covered with transparent Canadian Balsams to reduce the grinding traces on the otolith surface and to improve micro-increment discrimination. To prevent loss of micro-increments during the polishing process, the sections were frequently checked with a binocular microscope to avoid nucleus or excessive marginal grinding. Finally, the slides were dried at 100°C for 1 hour.

Readings and interpretation of daily micro-increment growth were carried out by transmitted visible light at 400x magnification. To avoid possible underestimation of

increment counts, several trials were performed at higher magnification (1000x) in the near otolith core zone. Thin sagittal sections were obtained as shown in Figure 4.5.2.

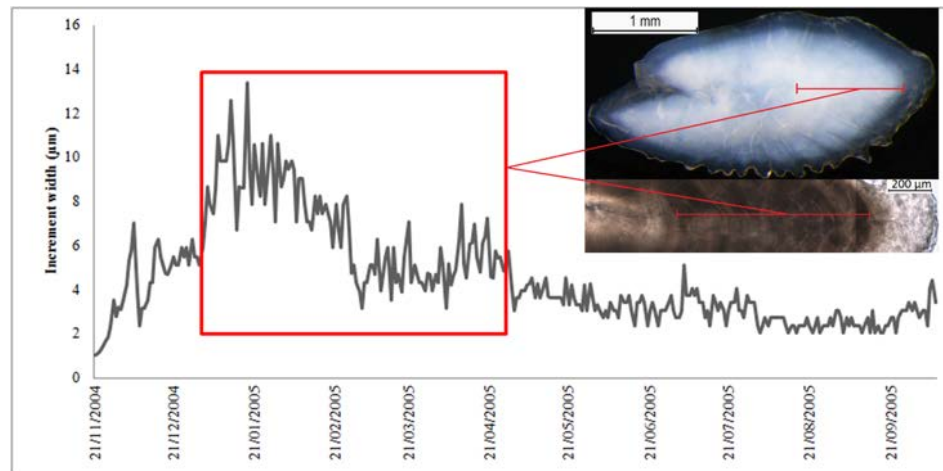


Figure 4.5.2. An example of whole otolith (magnification: 25x) image (scale bar: 1 mm) and its thin section (magnification: 400x) image (scale bar: 200 µm) with its nucleus and daily rings. In the two images a red bar indicates the FGP (*fast growth pattern*). Furthermore in the graph (corresponding to the daily growth pattern of the specimen) a red square indicates also the FGP zone.

Daily growth patterns

Micro-increment measurements were carried out on a selection of 10 random fish for each 0.5 cm size class was taken within the size range 5.5–12 cm, so daily increment width evolution and growth patterns were obtained by measuring a subsample of 89 individuals. The images were processed by an image-processing system following a transect from the core to the posterior margin of the otolith (Figure 4.5.2).

After the preparation and reading, and taking in account the birthdate month, further otoliths analyses were accomplished to corroborate the interpretation criteria of annual age reading by following the growth pattern changes along the seasons. Recently it has been observed that the opaque ring formation was a fast growing period (FGP), generally laid down during summer, while the hyaline band represents the slow growing winter period (Aldanondo *et al.*, 2016; Uriarte *et al.*, 2016). Taking into account the sudden observation the part of the otolith laid during the FGP represent the most important part of growth, during the first year of life. Therefore, a further measurement was restricted to such fast growth area defined as: the part of the growth increments wider than 6 microns which correspond to the bigger increment laid down during the slow growing period in the increment diagram, which corresponds to the wider opaque zone in the whole otolith and to the more shadowed area in the thin sections (Figure 4.5.2).

Habitat characterization

In order to investigate backward possible time integrated habitat effects on anchovy growth, satellite measurements of water temperature and primary production were obtained in the investigated areas starting from the catch date backward, covering the full first year of life from January 2004 until December 2005. These satellite data were clipped considering the 200 m isobath as end limit, in order to extract the environmental conditions related to the restricted habitat of juvenile anchovies over the continental shelf (up to 150 m depth) (Giannoulaki *et al.*, 2013), which displays a substantial overlap with spawning grounds and the main distribution area of anchovy (Giannoulaki *et al.*, 2013; Basilone *et al.*, 2013).

SST monthly statistics (the average value for each month/year in each area) were obtained by computing monthly mean maps from 1 km spatial resolution daily images, acquired from Copernicus Marine Environment Monitoring Service (<http://marine.copernicus.eu>). For Chl *a*, monthly mean maps were available for the considered period and no further processing was necessary.

Age, growth, and Birthdate distribution

The age of the younger examined fish was 52 days, corresponding to 55 mm TL (Figure 4.5.3).

While the older sampled individuals reached as much as 325 days at 123 mm TL. Therefore, it was not possible to detect a complete annual ring (defined as an opaque and a hyaline ring), however these data allowed to discard checks that may be confused as the true first annual ring (ICES, 2009).

The overall birthdate frequency distribution appeared strongly skewed toward July and August, while the older individuals were represented by few fish born in winter and spring (Figure 4.5.4).

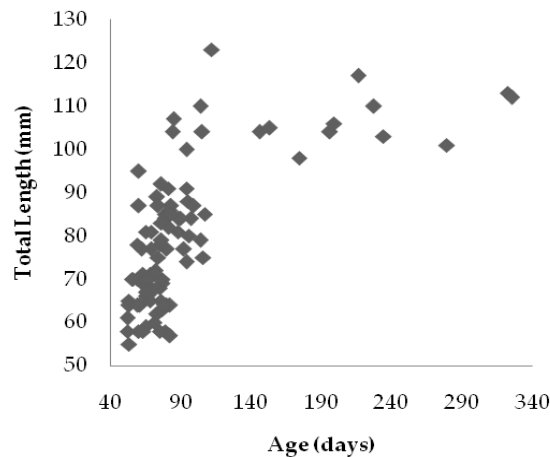


Figure 4.5.3. Relationships between total length (mm) and age in days. Regression coefficients: Intercept 60.77; slope 0.20; Pearson coefficient (r^2) 0.46.

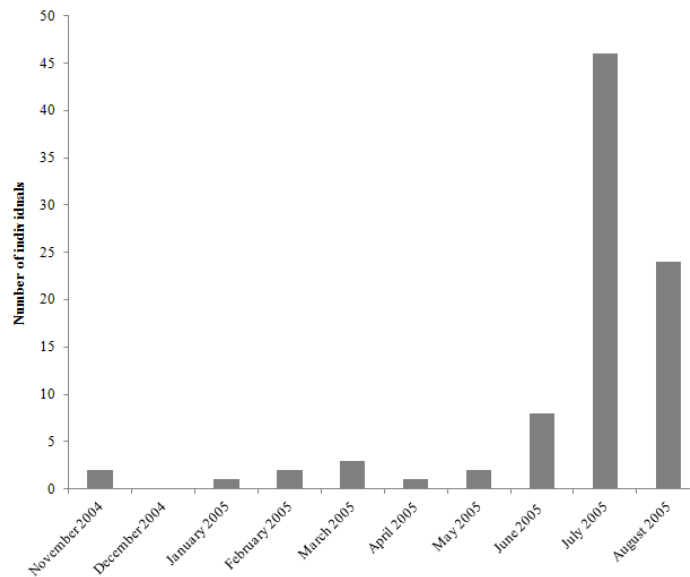


Figure 4.5.4. Birthdate frequency distribution of the 89 fish used for the present study.

Daily growth patterns

Data on otoliths averaged micro-increment evolution pattern showed a steady growth in the first 41 days, when it was reached its maximum increment width around 20 μm (Figure 4.5.5). Later, mean increments width decreased reaching a mean growth around 5 μm at about 110 days old, since then on continued to display a gradual reduction until reaching 3 μm at 240 days old. In the core area, micro-increment width starts with thin rings (around 5 μm) (Figure 4.5.5).

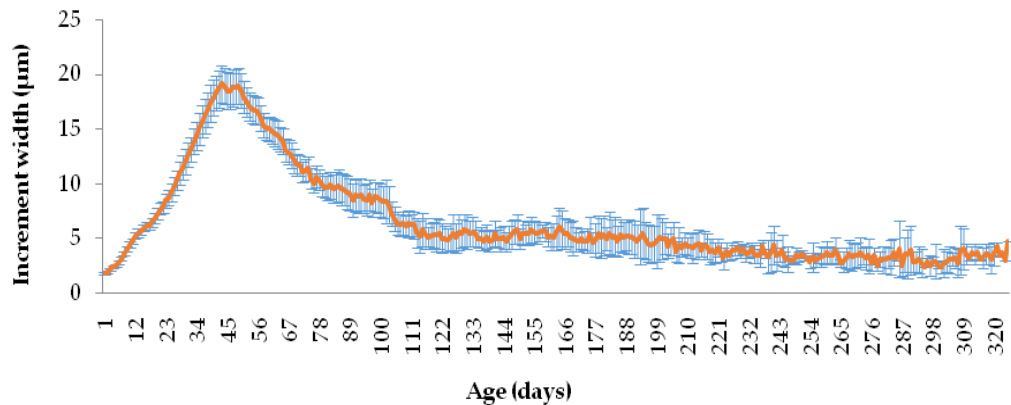


Figure 4.5.5. Micro-increment growth pattern obtained by daily ring measurements for the 89 selected individuals. Error bars refer to confidence intervals (95%).

The seasonal evolution of daily growth patterns, as indicated by the variability of width micro-increment averaged in the so-called FGP band, presented a clear increasing trend of FGP from November 2004 up to August 2005, according to the distance from the spawning peak (closer birthdate to the spawning peak displayed higher FGP) (Figure 4.5.6).

Birthdates split into month classes displayed a higher growth rate of the FGP formation along the year. Particularly, the increment width monthly patterns exhibited an increasing growth rate during the first 100 days as much as the summer months were approaching (Figure 4.5.6).

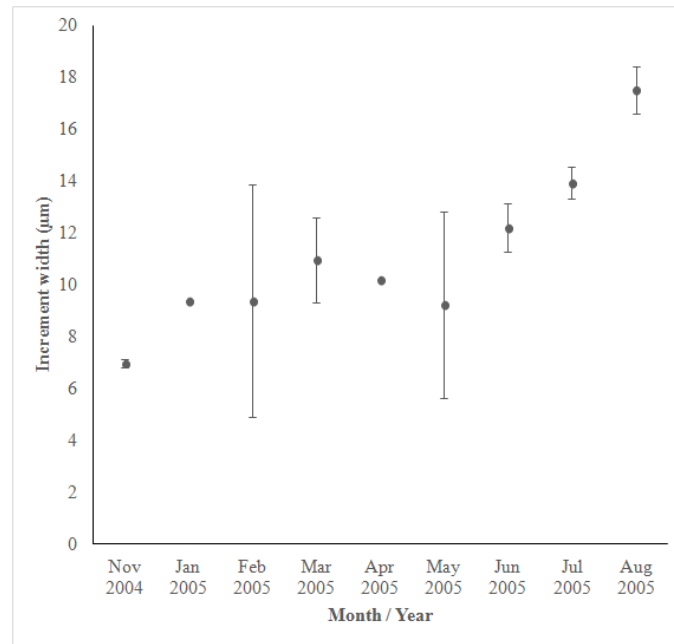


Figure 4.5.6. Average increment width during the fast growing period (opaque zone) related to the birthday of the analysed samples. Error bars refer to confidence intervals (95%).

Habitat condition

Comparing seasonal growth trend with environmental parameters monthly evolutions, very similar patterns were recorded between the SST and the average micro-increment width during the FGP (Figure 4.5.7a). Starting from January 2005 onward, a significant linear relationship arose between the two sudden variables ($r^2 = 0.78$; $p < 0.001$). On the contrary, no particular relationship was observed with the Chl *a* concentration (Figure 4.5.7b).

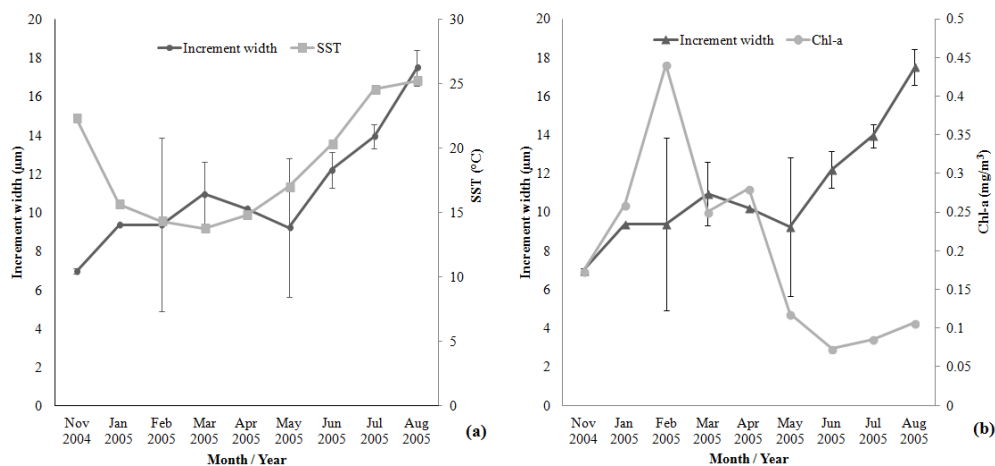


Figure 4.5.7. Evolution patterns of sea surface temperature (SST, °C) and concentration of chlorophyll *a* (Chl *a*, mg/m³) in the Strait of Sicily, in 2004 and 2005. Satellite data acquired from Copernicus Marine Environment Monitoring Service (<http://marine.copernicus.eu>).

Conclusions

Observed age range showed that the youngest individuals, collected during the survey, were at least 1.5 month old (52 days) while the oldest recorded fish was almost one year-old.

The back-calculated hatching-date distribution showed that the recruitment derived mainly from July-August, thus confirming the seasonal timing peak of the spawning period for anchovy in the study area (Basilone *et al.*, 2006 and 2015). Although very few specimens were recorded during autumn and winter period, the wide distribution of hatching dates of juveniles (9 months) reflects the protracted spawning period.

The otolith growth pattern appeared strongly supported by the microstructure morphology as well as the morphology of the whole otolith not sectioned. The translucent area represents a slow growing period (<6 micrometre) as indicated by the low average values of micro-increment in these zones. Furthermore, such zone in the sectioned otolith appears more transparent under transmitted light further supporting a weaker accumulation of calcium carbonate. On the contrary, during the fast growing period (FGP) in spring and summer, the otolith laid down a wide opaque zone lasting for 5-6 months. The position of the check, measured from the nucleus, gave results highly comparable with those obtained in other areas (ICES, 2013; Aldanondo *et al.*, 2016). The mean calculated measure of the first check in the present study is $899 \pm 61 \mu\text{m}$. Therefore, such measurements could be used as gauge to avoid misinterpretation of the true annual ring in case of not well defined growth pattern, although more analyses are recommended to obtain more robust results, specially increasing the number of observations on fish born during autumn, winter and spring.

The methodology applied in the present study allowed to obtain a well-defined growth pattern, also comparable with other studies, i.e. from the Bay of Biscay. Furthermore, present daily counts as well as the estimates of daily increment displayed patterns comparable to those attained in the Bay of Biscay and in Greek waters for the same species (Cermeño *et al.*, 2008; Schismenou *et al.*, 2013; Aldanondo *et al.*, 2016). The anchovy increment formation in the oldest individual (born in autumn 2004) displayed a slower growth in spring-summer period. Otherwise, for fish born later than February until June, the growth rate increases at an intermediate level, while it reaches the highest increment width in the fish born in August. Seasonal patterns evolution suggested a greater growth rate in the fish born close to the spawning peak in July-August for this area (Basilone *et al.*, 2006 and 2015). The width variability of FGP on otolith and, consequently, the modifications in body growth, according to the distance between birthdate and spawning peak of the target species, may be due to a different, adaptive fitness. Such observations propose that seasonal differences in growth performances would be due a faster growth period, which also reduces the anchovy mortality in a very critical window from larval up to recruitment phases.

The role of temperature in the anchovy population, inhabiting the study area, was considered relevant as an index of the goodness of the spawning site (García Lafuente *et al.*, 2002; Basilone *et al.*, 2013), or as a cue for the onset of the breeding season (Basilone *et al.*, 2006). This study showed how SST appears to play an important role also in the regulation of growth dynamics of the juvenile fish.

Fish born during the breeding season probably benefit from more favourable conditions for growth than fish born out of the reproduction peak. Such conditions may be linked to the higher SST and to the great stability of the water column, typical for the study area during summer (Bonanno *et al.*, 2014). A stable, well developed thermocline would have important effects on plankton dynamics.

Present observations did not highlight any significant effect of primary production, as proxy of food availability, on daily growth pattern of the analysed species.

Summarizing, present data are well consisting with the available literature existing in the European anchovy, both in Mediterranean and Atlantic waters (ICES, 2009; Cermeño *et al.*, 2008; Schismenou *et al.*, 2013; Aldanondo *et al.*, 2016).

4.8 Adriatic Sea

No validation/studies were carried out for this area.

4.9 Ionian Sea

Completed validation studies for anchovy in Greek Seas are lacking. Empirical rules such as the presence of a hyaline ring close to nucleus (probably a check), the use of measurements as well as age interpretation methods described by the previous workshop (ICES, 2009) can help the age reading of European anchovy. Taking into consideration the individual total length can be helpful in some cases but not in all. Thickness and clarity of translucent rings is not always enough to show the presence of a winter ring and misinterpretations can sometimes happen.

4.10 Aegean Sea

No validation/studies were carried out for this area

4.11 Tunisian area

This is a summary of Presentation 9: Adel Gaamour and Sana Khemiri (INSTM). Tunisian Waters anchovy (GSA 12, GSA13 and GSA14): Biological data and indirect validation studies.

We have presented the distribution of anchovy along the Tunisian costs (GSA 12, 13 and 14) and the main useful parameters to estimate the age of the species. For each GSA area the length at first sexual maturity and the reproduction period was established (Gaamour *et al.*, 2004a; Gaamour *et al.*, 2004b; Khemiri, 2006; Khemiri and Gaamour, 2009). In Tunisia, Anchovy is able to reproduce from April to October. The fork length at first sexual maturity (LF50) is 6.2, 7.6, and 8.3 cm respectively for GSA 14, 13, and 12. The common birth date considered is 15 July. The definition of the first winter growth mark is based on the analysis and estimation of micro-increment of otolith of 38 anchovies. The validation of age estimation is done by the analysis (quantitative and qualitative) of the marginal increment. We conclude that the species has one growth cycle per year and that the formations of the opaque annual mark occur from April to October/November. The age length key and growth curves and parameters of Van Bertalanffy was established (Khemiri, 2006; Khemiri *et al.*, 2007). We note that the growth is faster for offshore anchovies than that of inshore specimen. This seems to be related to the presence in the same area of two populations or groups of anchovies one sedentary in the inshore area and the second migratory (homing migration) between inshore and offshore area. This conclusion is supported by morphometric, genetic studies and by otolith microchemistry analysis (Borsa *et al.*, 2004; Borsa, 2002; Bouchnek-khelladi *et al.*, 2008; Khemiri, 2006; Khemiri *et al.*, 2014).

In many cases the interpretation of anchovy otoliths growth marks is ambiguous and especially the definition of the first winter mark. Also the winter hyaline zone may be composed of several close hyaline zones and may be weakly marked.

The validation of the first annulus is based on the analysis of daily increments of 38 specimens. The age of these anchovies range between 79 and 300 days (Khemiri and Gaamour, 2005; Khemiri, 2006).

To establish the chronology of deposition of growth marks we used the indirect validation method (marginal increment analysis), consisting of observing the quantitative and qualitative evolution of the marginal zone of the otolith over time:

- Qualitative data: This process consists of evaluating the presence or the absence of the mark at the edge of the otolith by the mean of the percentage of individual with marginal hyaline mark.
- Quantitative data: This analysis consists of measuring the distance separating the latest marks at the edge of the otolith. We used the relative marginal distance $RMD = (AMD)/(R_n - R_{n-1})$ with : AMD : Absolute marginal distance ; R : total radius of the otolith ; R_n : radius of the otolith at the last mark ; R_{n-1} : radius of the otolith before the last Mark

For the two parameters we observe one peak per year so the anchovy has one growth cycle per year along Tunisian coasts (Figure 4.11.1). The opaque zone is laid down from April to October/November and the translucent zone is deposited from December to March

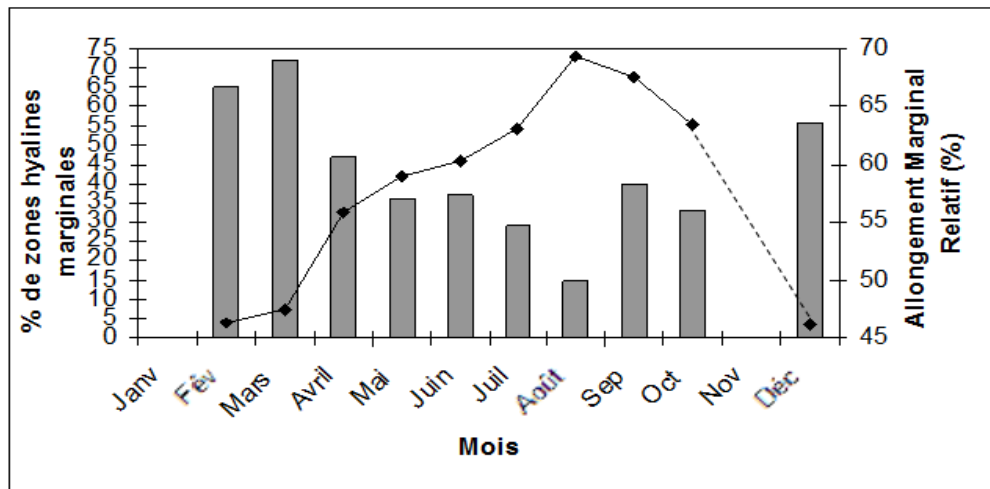


Figure 4.11.1. Monthly evolution of percentage of hyaline edge formation and relative marginal distance

4.12 North of Morocco

No validation/studies were carried out for this area.

5 Review of ageing techniques

5.1 Otoliths preparation techniques

Within the participating institutes the methods of treatment of the otoliths before reading are relatively well standardized. Most institutes are reading whole otoliths embedded in resin, and some, immersed in water or ethanoic solution (70%). (Table 5.1.1)

5.2 Otolith image processing techniques

All participant laboratories have a methodology to capture and analyse otoliths images. The type of capture/analysis software varies between laboratories, as well as the type of camera. Most laboratories use the otolith images for thesis and validation studies of anchovy and other species. Otoliths images can be available by all laboratories for otolith exchanges. The most frequent otolith measures are: otolith radius, annual ring radius and the distance between rings. JPEG and TIFF are the image format most commonly used. A summary of the otolith image processing techniques used by WKARA2 participant laboratories is shown in Table 5.2.1.

Table 5.1.1. Summary of anchovy otolith sampling methods and preparation techniques of each participant laboratory.

Country/Institute	Laboratory	Stock/area	Sample	Periodicity	Otolith sampling	Total N/Year	Otolith preparation
Spain-AZTI	Pasaia (Basque Country)	Bay of Biscay anchovy: VIIIc, VIIIb	Egg Surveys	Annual-Spring	60 by fishing haul	2000	Black plastic moulds and polyester resin (Eukitt).
			Acoustic Surveys	Annual-Autumn	Stratified by length class, 10 otolith pairs by length class (0.5 cm)	2500	
			Landings	Monthly	50 by haul	2000	
Spain-IEO	Santander/Vigo	Bay of Biscay anchovy: VIIIc, VIIIb	Acoustic Surveys	Annual-Spring	Random, 40 by fishing haul	300	Black plastic moulds and polyester resin (Eukitt). One plaque of each sample (10 pairs of otoliths) was stored dry for other studies (daily growth or microchemical studies) and immersed in water for observations
			Bottom trawl survey	Annual Autumn		300	
			Landings	Monthly (fortnightly in spring)	Random, 100 by sample	1500	
		Anchovy IXa: Galician waters-Subdiv. IXa North	Acoustic Surveys	Annual-Spring	Random, 40 by fishing haul	100	
			Landings	Monthly	Random, 100 by sample	400	
		Anchovy IXa: Gulf of Cadiz-Subdiv. IXa South	Acoustic Surveys	Annual Summer & Autumn	50 by haul, plus tails	about 2000	
			Landings	Fortnightly	100 random	about 2000	Black plastic moulds and polyester resin (Eukitt).
	Cadiz	Morocco: North Atlantic area (North of 34°18'00")	Landings	Monthly, with the exception of the close season (February-	Stratified by length class, 5 otolith pairs by length class (0.5 cm)	500	
						200	
	Málaga	GSA01: Northern Alboran Sea	Acoustic Surveys	Annual Summer		700	
			Landings	Monthly		700	
		GSA06: Western Mediterranean Sea	Acoustic Surveys	Annual Summer		700	
			Landings	Monthly		700	

Table 5.1.1. Cont.

Country/Institute	Laboratory	Stock/area	Sample	Periodicity	Otolith sampling	Total N/Year	Otolith preparation
Italy-IAMCR-CNR	U.O.S Capo Granitola (Sicily)	GSA16: Strait of Sicily	Acoustic Surveys	Annual Summer	Stratified by length class, 10 otolith pairs by length class (0.5 cm)	about 1500	Black plastic moulds and dried for observation
			Landings	Fortnightly		about 1500	
		GSA09: Northern Tyrrhenian Sea	Acoustic Surveys	Annual Summer		about 1500	
		GSA10: Southern Tyrrhenian Sea					
Italy-COISPA	Bari	GSA10:Southern Thyrrhenian	On bord observers and Market	Monthly	Stratified by length class and sex, 5 otolith pairs by length class (0.5 cm)	500	Stored dried in vials and immersed in sea water for observation
		GSA19: Western Ionian				500	
Croatia-IOF	Split	GSA 17: Northern and Central Adriatic Sea	Acoustic Surveys	Annual	Stratified by length class, 5 otolith pairs by length class (0.5 cm)	1000/1500	Stored dried in vials and immersed in alcohol for observation
			on board observers	Monthly		400	

Table 5.1.1. Cont.

Country/Institute	Laboratory	Stock/area	Sample	Periodicity	Otolith sampling	Total N/Year	Otolith preparation
Greece-ELGO	Kavala	GSA22: Aegean Sea	Surveys	Monthly	Stratified by length class, 12 otolith pairs by length class (0.5 cm)	500	Stored dried in vials and immersed in water for observation
			Landings				
Greece - HCMR	Athens	GSA22: Aegean Sea	Acoustic Surveys	Summer/autumn	Stratified by length class, 10 otolith pairs by length class (0.5 cm)	700	Stored dried in plastic cases and immersed in water for observation
			Landings	Monthly		500	
		GSA20: East Ionian Sea	Acoustic Surveys	Summer/autumn		400	
			Landings	Monthly		400	
Tunisia- INSTM	La Goulette	GSA 12 (North of tunisia), 13 (Gulf of Hammamet and 14 (Gulf of Gabes) : Tunisian coasts	Acoustic Surveys	Annual until 2009	Random 100/sample	1000/1500	Stored dried in envelopes and immersed in Essence of mint for observation
			Landings	Monthly	Random 100/sample	1000/1500	

Table 5.2.1. Summary of the otolith image processing techniques used by WKARA2 participant laboratories

Country/Institute	Laboratory	Image capture software	Type of camera	Magnification	Otoliths measures	Image format	Image uses
Spain-AZTI	Pasaia (Basque Country)	Olympus Cell Sens Dimension 1.6 Micro	Imaging Micropublisher 5.0	x20	Otolith total length; radius	JPEG/TIFF	Exchanges, otolith images collection
Spain-IEO	Santander	NIS-Elements Viewer 4.0	Color Digital Camera NIKON Digital Sigth. DS-5M	x20	Otolith diameter/radius, annual ring radius	LIM images, JPEG, TIFF, psp	Exchanges, thesis, validation studies, publications, IEO manuals, otolith images bank
	Cadiz	NIS-Elements AR 3.2	NIKON DXM1200C	X20	routine. Otolith diameter, and radii (post rostrum, antirostrum	JPEG2000, jpg (preferred)	Exchanges, validation studies, otolith images bank
	Malaga	Leica Application Suite (LAS) 4.1	Leica DC200	X25	Otolith diameter/radius, annual ring radius	JPEG	Exchanges, thesis, validation studies, otolith images collection, training
Italy-IAMCR-CNR	U.O.S. Capo Granitola (Sicily)	Leica Application Suite (LAS) 4.1	Leica DC200	X25, x32	Otolith diameter/radius, annual ring radius, checks radii	TIFF	Exchanges, thesis, validation studies, otolith images collection, training group

Table 5.2.1 Cont.

Country/Institute	Laboratory	Image capture software	Type of camera	Magnification	Otoliths measures	Image format	Image uses
Italy-COISPA	Bari	BEL Micoimage analyzer	Leica WILD M3C	X40	Otolith total length; radius length, annual rings radius length.	JPEG/TIFF	Exchanges, validation studies, publications, reference collection, intra lab ring test
Croatia-IOF	Split	Dino-lite software	Dino-Lite Digital microscope	/	Otolith total length and radius length, annual rings	JPEG	Exchanges, otolith images collection
Greece-ELGO	Kavala	Image Pro Plus ipwin 32	Color Digital Camera NIKON Digital Sighth. DS-Fi1	x20	Otolith diameter/radius, annual ring radius	JPEG images	Exchanges, publications, FRI Final Reports, otolith images bank
Greece-HCMR	Athens	Image Pro Plus	SONY WILD	x20, x25, x32	Otolith diameter/radius, annual ring radius	TIFF	Exchanges, otolith images bank
Tunisia- INSTM	La Goulette	Motic ImagesPlus 2.0 and TNPC 4.1	Moticam480		Otolith diameter/radius, annual ring radius	JPEG, TIFF	INSTM Project, thesis, validation studies, publication

6 Estimate (relative) accuracy and precision of anchovy, age determination in the main European fishing areas (ToR c)

The main results of the exchange of 2014 are presented in this part of the report (for more detail see in Villamor and Uriarte (2015) and Annex 4 of this report). A closer examination of the results of a new exercise was not realized during the Work-shop (see section 6.2).

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

6.1 Exchange 2014 results

The 2014 ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) identified the need of a full-scale European Anchovy (*Engraulis encrasicolus*) otolith exchange to take place in 2014 under the coordination of IEO and AZTI (Spain). It was the second exchange after the one of 2009 that anchovy otoliths of Atlantic and Mediterranean were included together.

A questionnaire was distributed among anchovy ageing institutes before the exchange to get an overview of potential participants and methods used and also information of anchovy stocks in different areas (biology, fisheries, etc.). Within the participating institutes the methods of treatment of the otoliths before reading are relatively well standardized. A total of 576 images of anchovy otoliths were selected and uploaded for analysing using the WebGR application, distributed in 10 sets from different anchovy distribution areas and stocks (English Channel, Bay of Biscay, Portugal coast and Gulf of Cadiz, Alboran Sea, Western Mediterranean, Gulf of Lion, Southern Tyrrhenian, Strait of Sicily, Western Ionian and Aegean Sea) (Figure 6.1.1).

Eighteen readers from eight institutes and six countries (France, Spain, Portugal, Italy, Slovenia, and Greece) were participated. From all readers nine readers have a long-time experience reading (>5 years) anchovy otoliths (experts); one was intermediate and eight trainees.

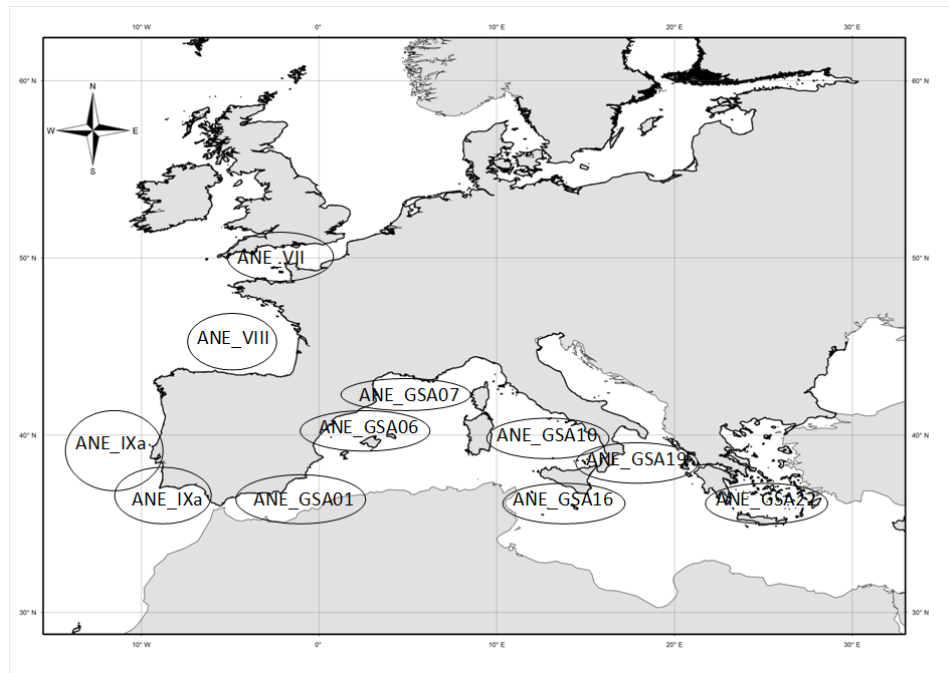


Figure 6.1.1. Collection areas of 2014 otolith exchange sample sets

Analyses were performed for the total areas and each area. For each area overall age reading were analysed and three additional analyses were performed: Analysis only with the expert group, analysis referring to intermediate and training group and analysis only with area readers in those areas where there were more than one reader (Bay of Biscay, 9a area, Strait of Sicily, Southern Tyrrhenian, Western Ionian and Aegean Sea) (Table 6.1.1 and Figure 6.1.2. For the total areas the average percentage of agreement (66%) and CV (58%) does not seem to be satisfactory. Most of the anchovy otoliths were not well classified by many of the readers during the 2014 exchange. By areas, the agreement with the modal age of all readers was low (between 59 and 74%) and CV was high (between 31 and 127%). In the case of the expert group, agreements and CV are highly variable, depending on the areas, showing the highest agreement in the area 7 and 8, with 80% agreement in both cases, and high variation of CV (73% and 22% respectively). In general, the results of the expert group improved compared to those of intermediate and training group in all areas, except in some areas of the Mediterranean (i.e. Western Mediterranean, Southern Tyrrhenian, and Ionian Sea). And the results of the area readers group are better (higher % agreement and lower CV) than the other groups of readers (including expert group), except for the area 9a (quite similar to respect expert group). This may mean that there are different criteria reading between areas, so that when comparing only the readers in their expertise area they are more precise because they all follow the same criteria reading. Analysis only done with the area readers group shows a higher overall agreement and low CV for Aegean Sea and Bay of Biscay readers (91% and 97% of agreement; CV of 11.4% and 6.7%, respectively). Possibly the success of the readers of the Bay of Biscay, compared with the other sets, is because exchanges and workshops have been conducted routinely since 1990 in this area, and there are sufficient criteria for the interpretation of anchovy otoliths. In the case of Aegean readers which show a great accuracy of its readings, both readers are of the same institute and therefore would have very consistent criteria.). Detailed results of 2014 otolith exchange are shown in Villamor and Uriarte (2015) and Annex 4 of this report.

Table 6.1.1 Summary of the average percentage of agreement, CV and relative bias by area y total.

% Agreement				
Set	All readers	Intermediate and Training readers	Expert readers	Area readers
Total	65.5%	66.7%	71.8%	
ANE_7	66.7%	60.9%	80.4%	
ANE_8	74.3%	71.3%	80.8%	90.9%
ANE_9.a	68.5%	65.4%	76.4%	75.7%
ANE_GSA01	58.9%	62.5%	63.5%	
ANE_GSA06	60.9%	66.2%	59.6%	
ANE_GSA07	73.4%	72.6%	75.1%	
ANE_GSA10	62.9%	68.9%	62.0%	67.3%
ANE_GSA16	58.5%	61.0%	59.9%	85.6%
ANE_GSA19	61.9%	68.9%	60.2%	73.5%
ANE_GSA22	70.0%	67.1%	78.3%	97.1%
CV				
Areas	All readers	Intermediate and Training readers	Expert readers	Area readers
Total	58.2%	48.6%	51.8%	
ANE_7	127.6%	90.0%	73.9%	
ANE_8	45.1%	44%	22.4%	11.4%
ANE_9.a	49.1%	43.9%	34.7%	33.0%
ANE_GSA01	58.7%	45.6%	71.1%	
ANE_GSA06	49.9%	38.5%	59.2%	
ANE_GSA07	31.3%	34.1%	30.3%	
ANE_GSA10	67.2%	51.2%	86.7%	58.1%
ANE_GSA16	78.7%	40.7%	73.8%	11.2%
ANE_GSA19	60.9%	50.1%	73.3%	55.3%
ANE_GSA22	55.7%	71.6%	42.8%	6.7%
Bias				
Areas	All readers	Intermediate and Training readers	Expert readers	Area readers
Total	0.11	0.16	0.08	
ANE_7	0.27	0.24	0.10	
ANE_8	0.11	0.17	0.00	0.03
ANE_9.a	0.21	0.15	0.18	0.04
ANE_GSA01	0.07	0.13	0.08	
ANE_GSA06	0.07	0.19	-0.01	
ANE_GSA07	0.06	0.19	0.10	
ANE_GSA10	0.10	0.11	0.06	-0.05
ANE_GSA16	0.26	0.26	0.18	-0.13
ANE_GSA19	-0.04	0.03	0.12	0.04
ANE_GSA22	0.06	0.20	0.04	-0.03

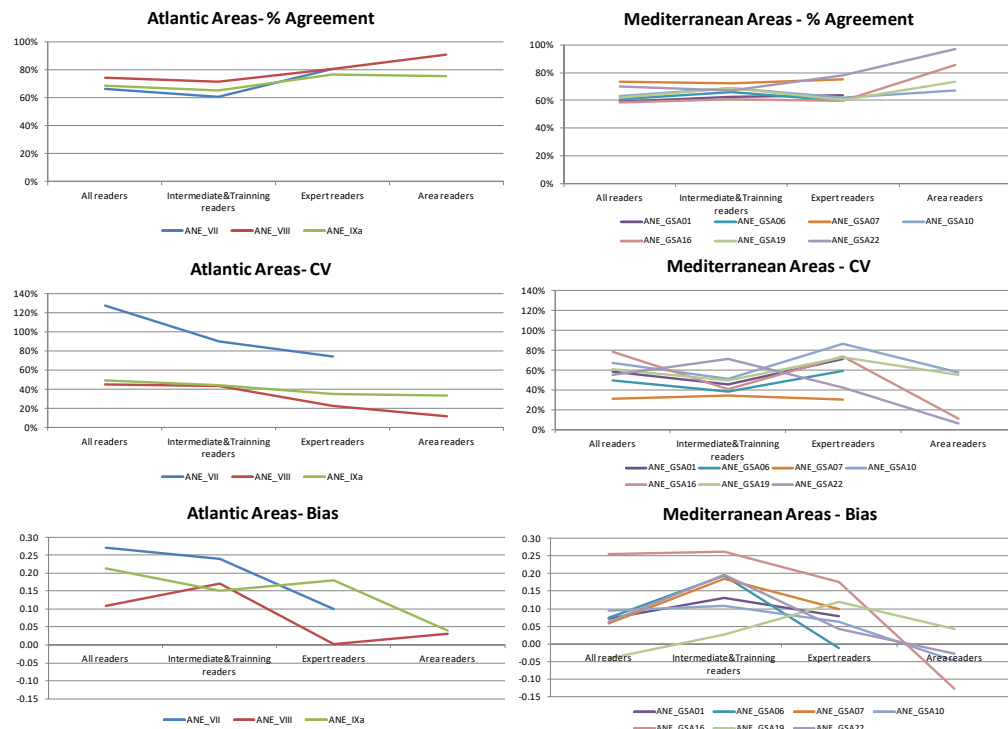


Figure 6.1.2 Summary of the average percentage of agreement, CV and relative bias by area.

Only 6 readers of the participants in the 2009 exchange and workshop were also participating in the current exchange of 18 participants. However, the results of the recent exchange show no decline of agreement but a slight improvement in all areas, especially in the 9a, and a significant improvement in the CV (lower variability) in all areas (Table 6.1.2).

Table 6.1.2. Comparison of the results obtained in the 2009 and 2014 exchanges in % of agreement in age determination and CV of the readings.

Set	Area	2009		2014	
		% Agreement	CV	% Agreement	CV
ANE_VIII	Bay of Biscay (all readers)	72%	85%	74%	45%
ANE_VIII	Bay of Biscay (BB readers)	89%	13%	91%	11%
ANE_IXa	Gulf of Cadiz& Portugal Coast	58%	68%	69%	49%
ANE_GSA01	Alboran Sea	61%	100%	59%	59%
ANE_GSA07	Gulf of Lion	72%	37%	73%	31%

The age compositions estimated by each age reader (Table 6.1.3) for the whole group show that some readers are interpreting the age structure of anchovy distinctly from the majority of readers. There seems to be a difference of criteria among some readers of the Mediterranean and the Atlantic areas. In particular there seems to be a difference of criteria among some readers (Mediterranean Readers 9, 10, 18, and 19 and Atlantic Reader 16) which tend to age older the fish than the rest of the readers.

Table 6.1.3. The age composition estimated by each reader and all age reader combined by areas and total. Cells in orange correspond to the Expert readers and green correspond to the Area readers.

Total areas																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	160	32	50	81	130	50	134	64	63	46	48	63	69	41	30	75	66	36	1238
1	294	329	343	277	340	294	374	254	207	291	320	296	315	268	195	342	185	89	5013
2	92	187	156	190	93	208	60	186	189	213	202	206	176	153	217	154	236	204	3124
3	25	26	24	27	13	21	7	50	73	25	6	8	16	41	116	5	81	197	761
4	-	-	1	-	-	-	-	5	10	-	-	-	-	2	15	-	6	46	85
5	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	1	5
0-5	571	574	574	575	576	573	575	559	543	575	576	575	576	505	576	576	574	573	10226
ANE VII																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	10	10	10	8	10	9	10	10	-	9	2	7	9	10	8	8	7	-	137
1	7	4	9	3	3	4	5	7	10	4	8	5	3	-	3	4	2	3	84
2	3	5	1	6	7	6	5	2	4	6	8	5	8	4	5	8	5	6	94
3	-	1	-	3	-	1	-	-	5	1	2	3	-	3	3	-	3	4	29
4	-	-	-	-	-	-	-	-	1	-	-	-	-	2	1	-	3	5	12
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
0-5	20	20	20	20	20	20	20	19	20	20	20	20	20	19	20	20	20	20	358
ANE VIII																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	5	5	5	7	6	4	6	6	4	5	7	8	6	3	7	6	6	2	98
1	29	25	30	19	29	30	38	13	12	31	21	20	28	17	24	24	13	4	407
2	21	27	27	35	26	22	20	32	40	22	41	41	31	27	27	35	33	11	518
3	15	13	8	9	9	12	6	17	13	12	1	1	5	11	12	5	17	40	206
4	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	12
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	70	70	70	70	70	68	70	68	70	70	70	70	70	58	70	70	70	69	1243
ANE IXa																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	7	5	5	11	6	8	6	6	6	7	4	7	6	7	5	7	2	-	112
1	72	72	56	56	66	51	77	36	45	45	48	44	60	23	23	66	19	12	871
2	12	14	27	25	20	32	9	34	28	40	39	39	26	40	49	21	52	34	541
3	1	-	3	-	-	1	-	15	13	-	1	1	-	12	12	-	14	36	109
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	8	9
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	92	92	91	92	92	92	92	91	92	92	92	91	92	81	92	92	92	92	1642
ANE GSA01																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	37	7	15	3	19	17	32	6	-	13	-	-	7	-	6	5	3	-	170
1	29	50	44	27	41	42	35	33	18	45	35	32	36	2	20	48	33	8	578
2	4	12	10	31	9	10	2	25	21	12	32	34	26	10	20	17	22	23	320
3	-	1	1	9	1	-	-	3	7	-	3	4	1	14	16	-	10	31	101
4	-	-	-	-	-	-	-	-	1	2	-	-	-	-	2	5	-	1	18
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	3
0-5	70	70	70	70	70	69	69	68	48	70	70	70	70	28	70	70	69	69	1190
ANE GSA06																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	23	1	-	2	26	-	29	1	-	-	-	-	-	6	-	-	-	-	91
1	29	27	39	41	30	39	28	33	15	42	31	36	39	37	11	36	15	4	532
2	7	27	19	17	4	20	3	22	22	17	28	24	21	14	27	24	37	28	361
3	1	2	2	-	-	1	-	1	11	1	1	-	-	1	19	-	7	25	72
4	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	1	3	8
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	60	60	60	60	60	60	60	57	49	60	60	60	60	58	60	60	60	60	1064
ANE GSA07																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3
1	8	19	17	19	28	15	24	15	20	15	30	25	25	15	19	23	20	3	340
2	23	18	18	17	10	22	14	22	16	20	8	13	13	21	15	14	14	18	296
3	7	1	2	1	-	1	-	1	2	2	-	-	-	1	4	-	2	16	40
4	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	38	38	38	38	38	38	38	38	38	37	38	38	38	37	38	38	38	37	681
ANE GSA10																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	32	1	8	15	33	10	25	22	5	10	9	12	9	8	2	12	11	2	226
1	18	32	34	18	11	24	24	19	26	23	31	28	28	44	31	30	16	18	455
2	4	19	12	21	11	21	6	13	16	22	15	15	18	3	8	13	18	21	256
3	-	3	1	-	-	-	-	1	8	-	-	-	-	-	13	-	9	14	49
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	54	55	55	54	55	55	55	55	55	55	55	55	55	55	55	55	55	54	987
ANE GSA16																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	23	9	1	6	15	8	16	7	7	7	6	6	7	6	7	8	7	5	151
1	26	31	33	30	36	24	44	17	12	23	35	33	25	34	6	38	5	4	456
2	13	19	25	22	12	30	5	24	24	29	25	25	24	23	18	20	33	15	386
3	1	6	6	8	3	4	1	12	16	7	-	2	10	1	30	-	19	25	151
4	-	-	-	-	-	-	-	4	6	-	-	-	-	-	5	-	2	16	33
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2
0-5	63	65	65	66	66	66	66	64	66	66	66	66	66	64	66	66	66	66	1179
ANE GSA19																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	26	1	10	11	21	3	20	14	9	4	5	6	8	11	1	9	11	4	173
1	21	31	28	28	34	31	35	25	29	38	28	32	31	31	25	36	25	19	614
2	7	20	16	22	-	20	-	13	21	19	14	17	16	14	20	10	17	22	268
3	-	-	1	-	-	1	-	-	3	3	-	-	-	-	1	9	-	2	30
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-5	54	54	55	55	55	55	55	52	55	55	55	55	55	55	55	55	55	55	985
ANE GSA22																			
Age	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	TOTAL
0	7	1	6	25	4	-	-	2	32	-	17	24	26	1	-	29	20	21	214
1	62	39	62	45	65	38	69	63	37	38	53	46	43	67	36	41	39	17	860
2	1	31	2	-	1	31	1	1	32	-	-	-	-	1	33	-	10	32	178
3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	1	-	3

The reasons that might explain the agreement and discrepancies appearing in the exchange may be: a) Difficulties in differentiating between true annual hyaline winter rings and false rings (or checks), b) Insufficient typical annual growth pattern recognition and insufficient criteria regarding the otolith edge that can be expected to be seen along the year. In addition it is observed that the different conventional birth dates between areas (in the Atlantic in January and in the Mediterranean in June or July) produces some difficulties for some readers (including expert readers) in determining the ages (mainly at ages 0) when the reader changes the conventional birthday which is accustomed.

There is analytical assessment for all anchovy stocks, except in the Areas 7, 9a and GSA10. In Division 9a and GSA10 no analytical assessment is made yet (trend based in qualitative assessment), but it is presumed that will be made in a short time. A special focus has to be devoted to the assessment of the anchovy stock in the GSA10 (Southern Thyrrenian) where the data are existing and theoretically enough to carry out a complete analytical stock assessment but important discrepancies arises during the last STECF meeting (https://stecf.jrc.ec.europa.eu/documents/43805/1517808/2016-12_STECF+16-22+-+MED+assessments+part+1_JRCxxx.pdf), where “quite scarce degree of consistency in age-class proportion between Catch-at-age data and MEDIAS survey samples appeared”, giving a warning on the reliability of the available age structure

The agreements and CVs between the readers contributing to the age structures inputting the assessments and the modal ages from the expert group readers for each stock are variables (Table 6.1.4). Major disagreements between the expert readers and the local area readers for stock assessment could be a matter of concern for the potential of hidden biases affecting the assessments and certainly would deserve further analysis. In general, under a few exceptions in some stocks, it seems that the experience of readers determines the interpretation they make of the otolith structure and the level of agreement achieved with the rest of expert readers.

It is therefore recommended, as far as possible, that only the age readings of the most expert readers are used for the assessment inputs and second that new readers pass a training processes from validated set of otoliths of the area they have to work with. Production of a collection of age validated otoliths by areas (or at least of agreed age determination by experts) is recommended for the purposes of helping in the training of new age readers. And finally it is also recommended to have regular exchanges, both internally and externally, to learn and to improve the agreements between readers across and within areas.

Table 6.1.4 Percentage of agreement and CV between the readers and modal age by stock. Modal age corresponds to the modal age of expert readers (expert readers in orange). Cells in green correspond to the assessment readers for each stock

Modal age of Expert Readers																			
Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader	Reader
1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	17	18	19		
ANE_VII: English Channel																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	85.0%	95.0%	75.0%	65.0%	95.0%	85.0%	95.0%	84.2%	5.0%	85.0%	30.0%	50.0%	85.0%	63.2%	60.0%	80.0%	45.0%	0.0%	65.6%
CV	11.0%	5.7%	10.5%	120.2%	8.0%	171.8%	6.7%	10.3%	10.8%	171.8%	33.0%	96.7%	165.8%	13.6%	122.8%	113.1%	103.3%	26.3%	127.6%
RANKING	4	1	6	12	2	6	2	5	14	6	15	12	9	11	15	10	17	18	
ANE_VIII: Bay of Biscay (in green B&B assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	90.0%	90.0%	84.3%	75.7%	90.0%	91.2%	75.7%	73.5%	51.4%	94.3%	54.3%	51.4%	82.9%	87.9%	74.3%	77.1%	57.1%	20.3%	73.3%
CV	10.3%	18.7%	21.0%	27.8%	16.9%	15.7%	18.8%	25.4%	39.7%	7.4%	30.6%	33.2%	20.8%	19.0%	35.6%	25.4%	33.3%	26.6%	45.1%
RANKING	1	3	8	7	5	3	11	13	18	1	14	15	10	6	11	9	16	17	
ANE IXa: Gulf of Cadiz & Portugal coast (in green IXa assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	91.3%	94.6%	73.9%	81.5%	94.6%	67.4%	93.5%	58.2%	52.2%	60.9%	60.9%	65.9%	85.9%	38.3%	38.0%	90.2%	33.7%	14.1%	66.6%
CV	36.7%	16.3%	51.9%	35.7%	18.6%	53.4%	5.2%	55.8%	46.9%	50.7%	38.6%	44.6%	42.3%	47.7%	51.1%	39.2%	47.6%	35.2%	48.9%
RANKING	4	1	10	5	2	11	3	16	13	12	8	9	7	15	18	6	17	14	
ANE GSA01: Alboran Sea (in green GSA01 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	57.1%	80.0%	51.4%	44.3%	81.4%	82.6%	60.9%	64.7%	27.1%	77.1%	50.0%	47.1%	61.4%	0.0%	30.0%	62.9%	49.3%	8.7%	54.3%
CV	71.8%	41.1%	53.1%	42.9%	108.3%	29.6%	61.6%	51.1%	36.1%	69.1%	33.3%	34.2%	45.7%	24.7%	52.6%	41.0%	44.7%	29.0%	58.7%
RANKING	13	2	8	15	5	1	11	6	15	4	8	10	6	15	18	3	12	13	
ANE GSA06: Western Mediterranean (in green GSA06 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	46.7%	68.3%	60.0%	86.7%	43.3%	80.0%	36.7%	73.7%	38.8%	88.3%	61.7%	65.0%	85.0%	51.7%	16.7%	81.7%	41.7%	6.7%	57.5%
CV	83.2%	42.9%	37.5%	30.3%	87.0%	27.4%	98.9%	32.8%	34.3%	20.2%	34.0%	34.5%	21.7%	50.8%	29.9%	25.5%	30.9%	24.1%	49.9%
RANKING	14	12	10	3	17	4	18	6	16	1	11	9	2	8	14	5	6	13	

Table 6.1.4. Cont.

Modal age of Expert Readers																			
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	
ANE GSA07: Gulf of Lion (in green GSA07 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	63.2%	92.1%	81.6%	86.8%	71.1%	92.1%	76.3%	71.1%	39.5%	89.2%	60.5%	73.7%	78.9%	78.4%	78.9%	68.4%	63.2%	40.5%	72.5%
CV	31.4%	10.9%	29.8%	24.2%	19.2%	19.2%	27.2%	30.5%	39.7%	21.6%	30.3%	28.1%	17.0%	28.5%	26.2%	35.9%	43.0%	26.6%	31.3%
RANKING	18	1	8	3	9	2	9	12	14	5	17	11	6	7	3	15	12	16	
ANE GSA10: Southern Thyrrenian																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	57.4%	49.1%	60.0%	83.3%	67.3%	65.5%	43.6%	80.0%	45.5%	63.6%	63.6%	69.1%	72.7%	50.9%	32.7%	76.4%	51.9%	14.5%	58.2%
CV	57.4%	26.5%	55.1%	82.6%	60.5%	48.4%	108.5%	116.7%	42.0%	48.5%	54.0%	65.9%	48.7%	46.8%	32.6%	60.7%	64.7%	29.8%	67.2%
RANKING	14	11	9	4	12	4	18	4	13	9	7	8	3	1	15	1	17	16	
ANE GSA16: Strait of Sicily (in green GSA16 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	58.7%	75.4%	48.5%	63.6%	74.2%	60.6%	66.7%	54.7%	36.4%	63.6%	68.2%	66.7%	66.7%	50.0%	27.3%	81.8%	34.8%	12.1%	56.1%
CV	66.0%	74.4%	48.1%	53.6%	39.3%	57.1%	37.3%	63.1%	57.1%	53.0%	47.4%	50.4%	57.9%	51.9%	52.8%	50.1%	52.2%	39.2%	78.7%
RANKING	14	7	8	8	3	11	5	16	18	10	2	4	12	6	17	1	14	13	
ANE GSA19: Western Ionian (in green GSA19 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	55.6%	51.9%	43.6%	65.5%	72.7%	50.9%	52.7%	88.5%	56.4%	43.6%	69.1%	58.2%	67.3%	34.5%	30.9%	65.5%	61.8%	27.3%	55.2%
CV	158.9%	19.9%	64.8%	69.3%	28.1%	36.6%	77.1%	121.4%	57.4%	39.9%	41.8%	48.2%	52.2%	61.7%	36.7%	56.3%	70.1%	38.7%	60.9%
RANKING	15	7	9	9	1	8	9	5	14	16	4	6	2	9	17	2	13	18	
ANE GSA22: Aegean Sea (in green GSA22 assessment readers)																			
MODAL experts	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	ALL
% agr.	90.0%	54.3%	92.9%	65.7%	97.1%	51.4%	94.3%	100.0%	57.1%	52.9%	77.1%	67.1%	65.7%	98.6%	48.6%	60.0%	60.0%	25.7%	69.8%
CV	30.1%	33.1%	27.2%	69.7%	16.9%	32.9%	11.5%	0.0%	86.8%	32.9%	51.8%	67.4%	72.0%	4.1%	32.3%	79.2%	72.4%	75.0%	55.7%
RANKING	6	12	5	9	3	14	4	1	18	12	7	8	11	2	16	14	9	16	

6.2 WKARA2 Exercise

During the workshop no formal exercise of otolith age readings for a new evaluation of agreement, bias and precision was carried out because since the beginning of the meeting it became evident that there were too large discrepancies in otolith interpretation and age determination across the different areas as to directly enter into such exercise. Furthermore attendees had doubts regarding the applicability to all areas of the rules for age determination found through validation studies in some particular areas (as in the Bay of Biscay). As a result of this, most of the work carried out during the workshop consisted in common sessions with all attendees going area by area through several otolith case examples to examine and jointly discuss the different otolith interpretations and allocation of ages which resulted in the 2014 exchange, or going through some new selected otoliths provided by the area experts.

The joint exercise of otolith interpretation and age reading was considered by all workshop attendees as to be of high interest and productive in achieving a greater degree of common interpretation and assignation of ages. Furthermore at the end of the exercise attendees had the impression that differences in growth patterns among areas were not as relevant as originally presumed. In addition it was noticed that some of the differences in age allocations were due to a wrong application of Rules corresponding to the birth date convention, particularly for the birthdate around the middle of the year.

7 Identify causes of age determination error (ToR c)

7.1 Common Causes of age determination error identified during the WKARA2 meeting from the 2014 Exchange

A detailed analysis of the discrepancies between readers in otolith interpretation from Exchange 2014 was carried out prior of the workshop and presented and discussed during the WKARA2 (Villamor *et al.*, 2016 Presentation):

First we quantified and tabulated the differences in age determination by:

- Checking the age range for each otolith by area;
- Calculating the maximum difference of the allocated ages by otoliths and areas;
- Counting and tabulating the number of otoliths for each difference in ages by area.

Next we try to identify the possible causes of age differences by area: Discrepancies in the position of the annual rings (with or without implications in modal age) by area, through the examination of several pictures of selected examples:

- One figure as an example for a 100% or >80% agreement,
- Other figure as an example for a medium high agreement (60–74%)
- Third figure are examples for low agreements (<50%);
- The other figures were chosen to show otoliths with a modal age given by the area readers group, not being endorsed by the most experienced readers of other areas

A synopsis of the analysis and discussions follows:

The results showed that the percentage differences in the age range were very high (Table 7.1.1). Most of the otoliths diverged in age determination by 2 or more ages.

Table 7.1.1 Percentage of otoliths by areas according to the range of ages in dispute between the age determinations made during the 2014 exchange exercise.

Difference age range	English Channel	Bay of Biscay	Division Ixa	Alboran Sea	Western Mediterranean	Gulf of Lion	Southern Tyrrhenian	Strait of Sicily	Western Ionian	Aegean Sea
0 age	0%	3%	1%	0%	0%	8%	0%	0%	0%	0%
1 age	15%	37%	37%	11%	13%	45%	44%	18%	47%	94%
2 age	45%	51%	47%	40%	46%	47%	55%	38%	49%	6%
3 age	30%	9%	15%	43%	49%	0%	2%	42%	4%	0%
4 age	10%	0%	0%	6%	0%	0%	0%	2%	0%	0%

These discrepancies arise when different readers mark annual growth rings in different locations of the otolith and can be divided in two groups depending on the effect in modal age:

- Discrepancies in the position of the annual rings with implications in modal age. These discrepancies take place when two or more readers record different number of annual rings, therefore providing different age estimates. These differences are easy to detect and were analysed in Section 7.
- Discrepancies in the position of the annual rings without implication for age determination. In this case, two or more readers have detected the same number of winter rings, but located them in different positions in the otolith. This type of discrepancies is not detectable when we are analysing individual age determinations estimated by different readers

To explore the reasons of the differences originated between readers (with or without impact on the modal age), we analysed the position of the annual winter rings in a selection of images by area from the exchange (See Villamor *et al.*, 2016 Presentation No.11).

The most common causes of age determination error identified were:

- Difficulties in differentiating between true annual winter hyaline rings and false rings (or checks), mainly the first annual winter ring;
- Insufficient typical annual growth pattern recognition and insufficient criteria regarding the otolith edge that can be expected to be seen along the year;
- Difficulties in recognizing the opaque or hyaline nature of the edge which may affect the age determination. Identifying hyaline edges seems to be a difficult issue as the edge continuously changes and no clear unambiguous definition exists;
- In addition it is observed that the different conventional birth dates between areas (in the Atlantic in January and in the Mediterranean generally in June or July) produces some difficulties for some readers (including expert readers) in determining the ages when the reader changes the conventional birthday he is familiar with;
- Difficulties in the application of the rule in the cases of the birthday date in the middle of the year (June or July), even for regular readers using this birthday.

After discussing and recognizing the reasons for the discrepancies, the following conclusions were reached for the interpretation of an otolith of anchovy:

- Try not to look at the size of the fish: see the structure of the otolith and growth pattern;
- Next try to interpret the otolith: What winter hyaline rings can be recognized resulting in a coherent growth pattern? How much has the edge grown throughout the year until its capture? Do the resulting annual growth pattern and edge formation match with known pattern of otolith growth and seasonality of edge formation by ages respectively?
- If a coherent interpretation is achieved then apply the age allocation rule corresponding to the adopted birthdate for the population, if not try another interpretation or discard the otolith.
- For the application of the ageing rules, it is compulsory to use the number of winter translucent rings recognized (after interpretation), rather than the total number of hyaline marks seen (which may include some checks).

Images detailing the discrepancies by area:

In the following example the continuous green arrows over the pictures point to true winter rings (discontinuous arrows are marks not being counted for age determination) and red arrows correspond to interpretations not selected by the workshop.

a) English Channel anchovy JC_14_TRIM3_CAMANOC_O_0029.jpg

Workshop agreement: 1-year old (correct interpretation it the one pointed at the left Top picture, well marked A1 plus Opaque wide border; certainly C08 and A15 checks are also visible). Workshop agreement contradicts the modal age produced in the 2014 exchange, but coincides with the modal interpretation given by the expert readers.

English Channel

Age Reading for anchovy JC_14_TRIM3_CAMANOC_O_0029.jpg,
18 cm, female, caught in September 2014
Conventional birthdates: 1st January



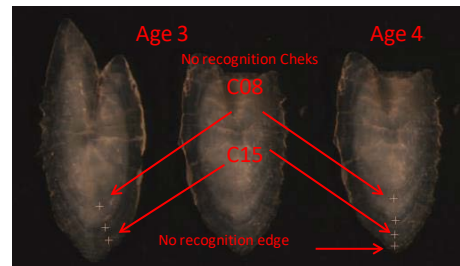
Problems identification:

- Annual Growth pattern
- True Annual ring/checks
- Edge or confusion with the conventional birthdates

39 % agreement Age 2

- Readings: 1-4 years
- 11 Readers not agree with modal age (7 Expert reader)

• Modal age 1 for Expert Readers

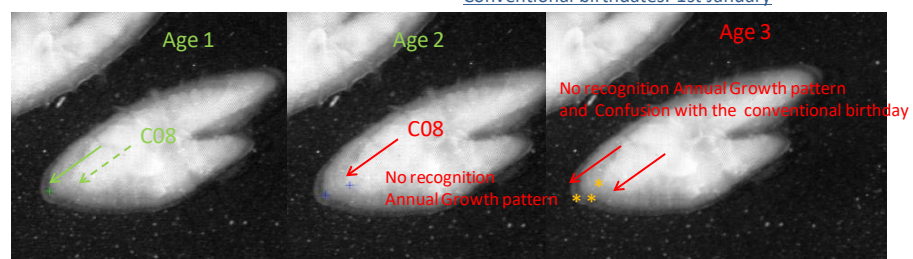


b) Bay of Biscay anchovy r5020b8.jpg,

Workshop agreement: 1 year old (correct interpretation as pointed in left picture). As pointed out by the work of Aldanondo *et al.* (2016) the range between C08 and final winter hyaline zone would correspond to the period of late autumn and winter. This workshop interpretation is the one given by the area readers but contradicts the modal interpretation given by all readers in the 2014 exchange.

Bay of Biscay

Age Reading for anchovy r5020b8.jpg,
15.0 cm, female, caught March 2013,
Conventional birthdates: 1st January



67 % agreement Age 2

- Readings: 1-3 years
- 6 Readers not agree with modal age (4 expert readers)

• Modal Age 1 for Area Readers

• Modal age 2 for Expert Readers

Problems identification:

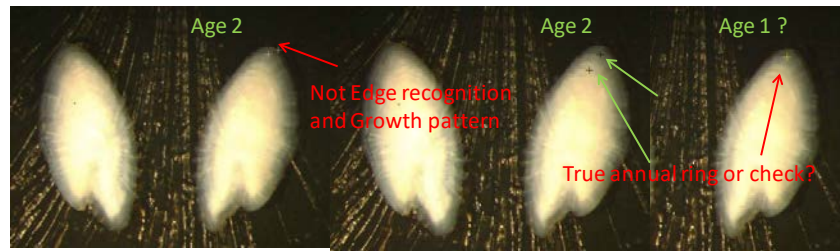
- Annual Growth pattern
- First Annual ring/checks
- Edge (conventional birthdates)

c) Division 9a anchovy IPMA_ANEIXaCN_7A.jpg,

No firm Workshop agreement: No well-marked winter hyaline ring. This otolith is a good example of a difficult otolith in this area. This is a candidate for micro-increments analysis in future to solve these doubts.

Division IXa

Age Reading for anchovy IPMA_ANEIXaCN_7A.jpg:
14.5 cm, female, caught May 2013,
Conventional birthdates: 1st January



56 % agreement Age 2

• Readings: 1-3 years

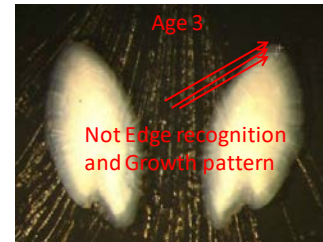
• 8 Readers not agree with modal age (5 Expert reader)

• Modal Age 2 for Area Readers

• Modal age 1 for Expert Readers

Problems identification:

- Annual Growth pattern
- First Annual ring/checks
- Edge recognition



d) Alboran sea anchovy 08082013-013.jpg,

No firm Workshop agreement: No well-marked winter hyaline ring. Modal age 1, but doubts about a potential age 0 (others discarded). Good Candidate for micro-increments analysis (Age 0 would correspond to an interpretation where the weak hyaline marks before the edge would be considered as checks prior to first winter mark).

Alboran Sea- GSA01

Age Reading for anchovy 08082013-013.jpg:
13.7 cm, female, caught August 2013
Conventional birthdates: 1st July



71 % agreement Age 1

• Readings: 0-3 years

• 5 Readers not agree with modal age (1 Expert reader)

Problems identification:

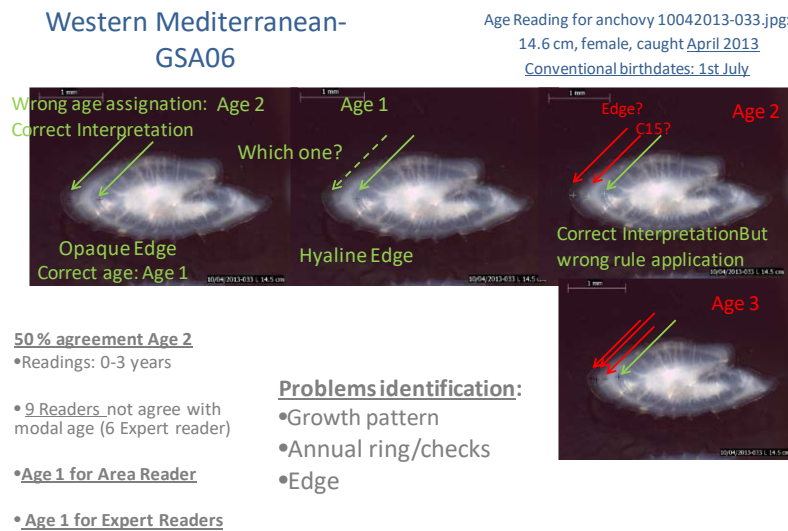
- Growth pattern
- Edge
- First Annual ring/checks



e) Western Mediterranean anchovy 10042013-033.jpg,

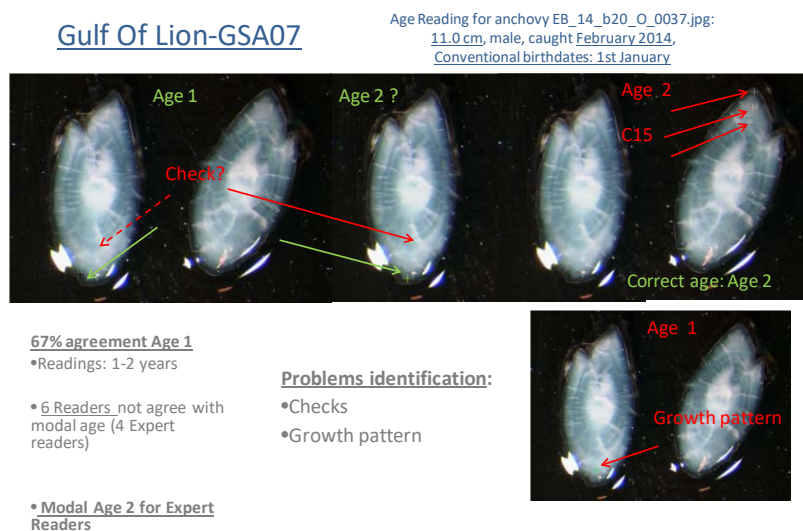
Workshop agreement: Age 1 (Two winter rings plus some opaque growth, as top left picture; notice and in the left picture for instance there was originally a bad application of the rule; instead of being 2-years old it is a 1-year old because of its birthdate in July,

so that age for the first half of the year equals the number of winter ring minus one). Notice as well that there is a wide first winter zone as pointed by Aldanondo *et al.*, 2016).



f) Gulf of Lion Anchovy EB_14_b20_O_0037.jpg,

Workshop agreement: 2-year old (Two winter rings; A1 + C15 + A2 at the edge; as birthdate is January 1st age equals the number of winter rings including the one formed or being formed during the first half of the year; so age = 2; as top right picture) (there is a wide first winter zone as pointed by Aldanondo *et al.*, 2016 with multiple marks). Notice that R1 size rule may lead to interpret A1 as a check, while it should prevail the visual interpretation of a correct wide winter ring matching the general growth pattern).



g) Southern Tyrrhenian Anchovy Reading GSA10_53.jpg,

Workshop agreement: Age 0 (One winter rings; A1 + opaque growth + semi-hyaline edge –C15? as left picture). Central and Right pictures exemplified correct otolith interpretation but wrong rule application for the birthdate in July (among others, this affected to

the area readers). Workshop agreement contradicts the modal age produced in the 2014 exchange, but coincides with the modal interpretation given by the expert readers.

Southern Tyrrhenian- GSA10

Age Reading GSA10_53.jpg:
9.5 cm, female, caught May 2012,
Conventional birthdates: 1st July



56% agreement Age 1

- Readings: 0-1 years
- 8 Readers not agree with modal age (3 Expert readers)

• Modal Age 0 for Expert Readers

• Modal Age 1 for Area Readers

Problems identification:

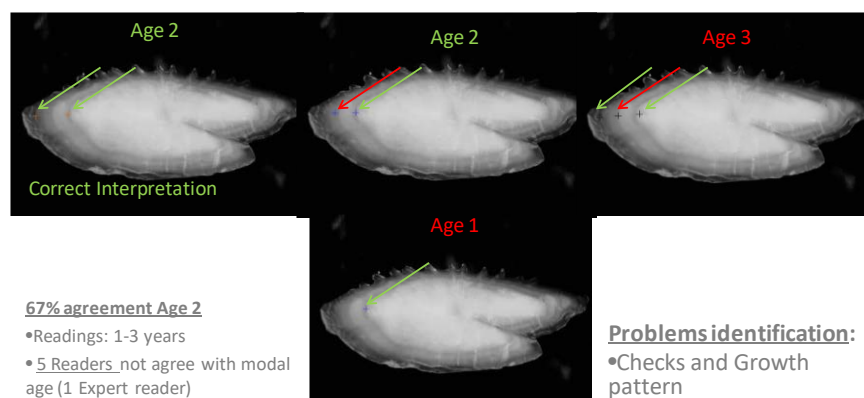
- Checks
- Edge or confusion with conventional birthday

h) Southern Tyrrhenian Anchovy Reading GSA10_10.jpg,

Workshop agreement: Age 2 (Two winter rings; A1 + C15+ A2+opaque growth on the left picture). Central and Right pictures exemplified wrong otolith interpretation. The are readers assigning it to age 3, by counting every mark as true winter ring, while most of the expert readers admitted the occurrence of check C15 .

Southern Tyrrhenian- GSA10

Age Reading GSA10_10.jpg:
14.0 cm, male, caught August 2013,
Conventional birthdates: 1st July



67% agreement Age 2

- Readings: 1-3 years
- 5 Readers not agree with modal age (1 Expert reader)

• Modal Age 2 for Expert Readers

• Modal Age 3 for Area Readers

Problems identification:

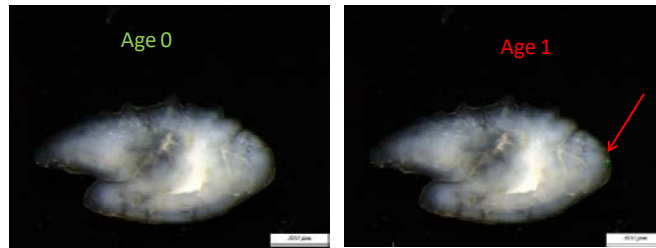
- Checks and Growth pattern
- Edge or confusion with conventional birthday

i) Strait of Sicily CB2010(10-5-10)_15(40x).jpg,

Workshop agreement: Age 0; assuming that the edge is hyaline and will become a full winter ring in the next months as the otolith resume opaque growth of the year. Controversial otolith, because it does not show a clear winter hyaline ring but perhaps at the edge in the rostrum and antistrostrum (and of little intensity in the postrostrum).

Strait of Sicily-GSA16

Age Reading CB2010(10-5-10)_15(40x).jpg:
 9.8 cm, undefined, caught May 2010,
 Conventional birthdates: 1st July



94% agreement Age 0

• Readings: 0-1 years

• 1 Readers not agree
 with modal age (Expert
 reader)

Problems identification:

• Edge or confusion with
 conventional birthday

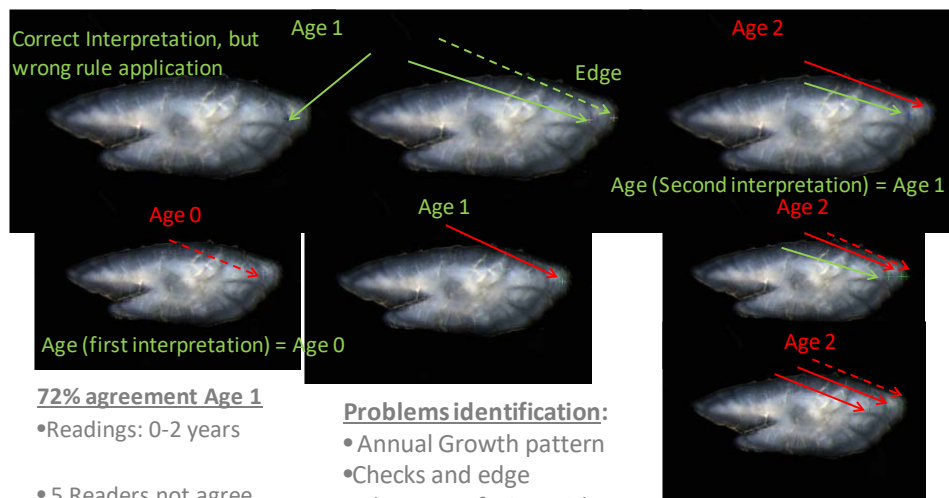
Doubtful: The Alternative interpretation would be that this fish is born in the current year during winter (age -1?) If these otoliths are common then moving the birthdate to January would help in solving coherent naming and grouping of year classes throughout the year. This is a good candidate for micro-increment analysis to clarify when these fish were born.

j) Strait of Sicily CB2010(13-4-10)_5(32x).jpg,

No Workshop agreement. First alternative: Age 0 (One winter rings; A1 + opaque growth + semi-hyaline edge -C15? as top left and central picture bottom left picture). Then $\text{Age} = \text{WR} - 1 = 1 - 1 = 0$. Second alternative: Age 1 interpreting there are two winter rings (A1+opaque1+hyalineEdge corresponding to second winter ring -A2-) (corresponds to the top right picture). Then $\text{Age} = \text{WR} - 1 = 2 - 1 = 1$. This is a good candidate to analyse by micro-increment analysis to verify if this otolith has already passed one or two winters (being either Age 0 or 1 respectively).

Strait of Sicily-GSA16

Age Reading CB2010(13-4-10)_5(32x).jpg:
13.1 cm, male, caught April 2010,
Conventional birthdates: 1st July



72% agreement Age 1

- Readings: 0-2 years
- 5 Readers not agree with modal age (4 Expert readers)

Problems identification:

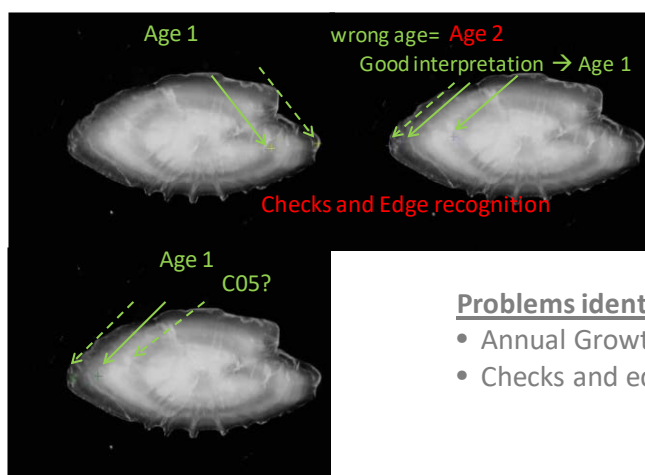
- Annual Growth pattern
- Checks and edge
- Edge or confusion with conventional birthday

k) Western Ionian anchovy GSA19_35.jpg,

Workshop agreement: Age 1: Two winter hyaline rings + narrow opaque edge (arrows are correct in the top right figure but age allocation is wrongly established. It should be an Age 1 fish according to the birthdate of 1 July.

Western Ionian-GSA19

Age Reading GSA19_35.jpg:
13.0 cm, female, caught May 2012,
Conventional birthdates: 1st July



83% agreement Age 1

- Readings: 1-2 years
- 3 Readers not agree with modal age (all Expert readers)

Problems identification:

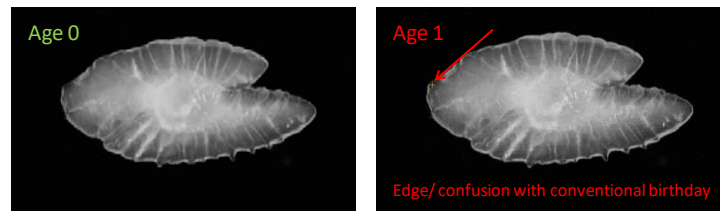
- Annual Growth pattern
- Checks and edge

l) Western Ionian anchovy GSA19_11.jpg,

Workshop agreement: Age 0: There is no winter ring; therefore age 0. (Correct interpretation and age allocation in the top left figure).

Western Ionian-GSA19

Age Reading GSA19_11.jpg:
8.5 cm, undefined, caught September 2013,
Conventional birthdates: 1st July



72% agreement Age 0

- Readings: 0-1 years
- 5 Readers not agree with modal age (3 Expert readers)

Problems identification:

- Edge/ confusion with conventional birthday

m) Western Ionian anchovy GSA19_28.jpg,

Workshop agreement: Age 1 (A1 + OW corresponding to central picture in the figure).
Difficult; Not much growth till sept and weak A1 ring.

Western Ionian-GSA19

Age Reading GSA19_28.jpg:
13.5 cm, female, caught September 2012,
Conventional birthdates: 1st July



50% agreement Age 2

- Readings: 1-3 years
- 9 Readers not agree with modal age (6 Expert readers)
- Modal Age 2 for Area Readers
- Modal Age 1 for Expert Readers

Problems identification:

- Edge/ confusion with conventional birthday
- Annual growth Pattern
- Checks

n) Aegean Sea Anchovy ANE20062014_1_18.jpg,

Workshop agreement: on Left picture interpretation. No clear winter ring. Age 1, it is in June (after birthdate) (A1 + OW). Difficult and controversial interpretation: very weak A1 ring; doubts if it is a real winter ring or just a check of an Age 0 individual. This is a good

candidate to analyse by micro-increment analysis to verify if this otolith has already passed none or one winter (being either Age 0 or age 1 respectively).

Aegean Sea-GSA22

Age Reading ANE20062014_1_18.jpg:
10.5 cm, male, caught June 2014,
Conventional birthdates: 1st June



72% agreement Age 1

•Readings: 0-1 years

• 5 Readers not agree with modal age (2 Expert reader)

• Modal Age 0 for Area Readers

• Modal Age 1 for Expert Readers

Problems identification:

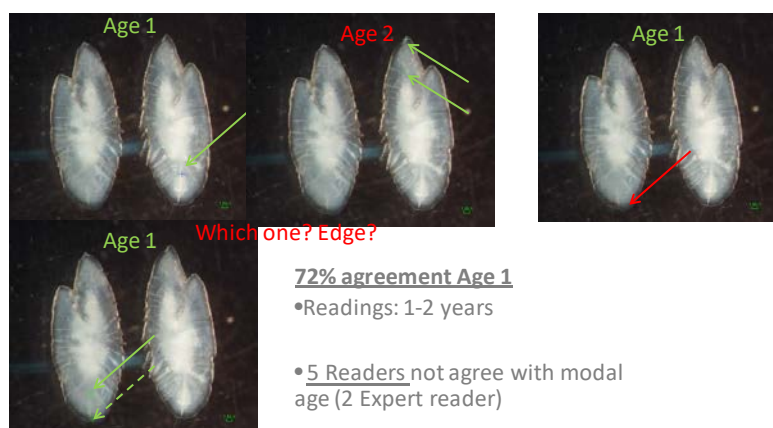
- Annual growth pattern
- Edge/ confusion with conventional birthday?

o) Aegean Sea Anchovy ANE20062014_1_18.jpg,

Workshop agreement: on Left picture interpretation. No clear winter ring. Age 1 (A1+ a far wider opaque growth band - than former one-- and partly hyaline edge). Then Age = pastWR = 1. The correct interpretation would be the top and bottom left pictures). This example supports the interpretation selected during the workshop to the former example above.

Aegean Sea-GSA22

Age Reading ANE25092014_4_08.jpg:
12.6 cm, female, caught September 2014,
Conventional birthdates: 1st June



72% agreement Age 1

•Readings: 1-2 years

• 5 Readers not agree with modal age (2 Expert reader)

Problems identification:

- Annual growth Pattern
- Edge/ confusion with conventional birthday

7.2 Examples of age determination by areas and follow up actions for future re-search on some otoliths

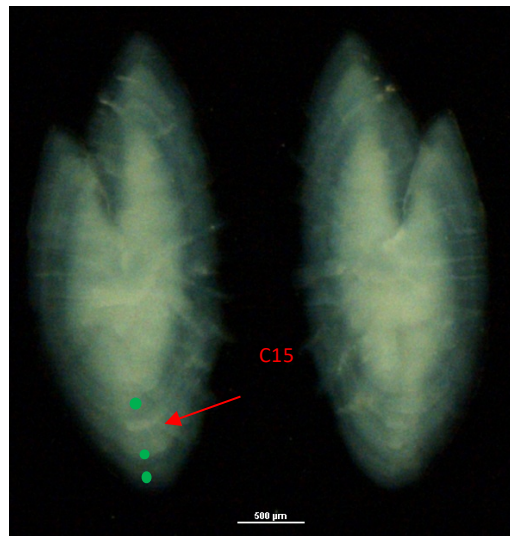
The following sections were reported by the area expert readers and the format of the contents are not entirely parallel for the different subsections. The Age readings were proposed by the area readers and agreed by the Workshop adding points for discussions and follow up actions for the most doubtful or difficult otoliths.

7.2.1 Bay of Biscay

Conventional Birthdate: 1 January

Below, they are displayed otolith types from 8c and 8b areas.

- Otolith 1: 1st semester



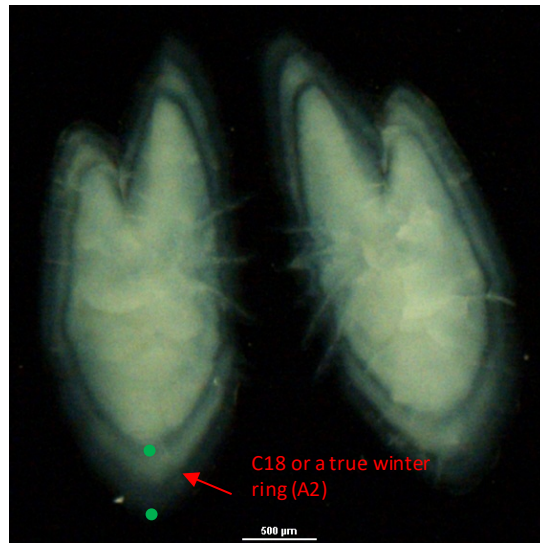
Date of capture: March 16, 2016,

Length: 161 mm.

Age determination: 3-years old

Otolith Interpretation: A1: 1st annual triple winter ring + spawning ring C15 (red arrow) + A2: 2nd marked annual winter ring + A3 at the edge (hyaline) ring.

Problems identification: triple winter ring, there is one equidistan ring between de 1st winter ring and the 2nd annual ring, then spawning ring C 15, because the ring it is in the middle.

- Otolith 2: 1st semester

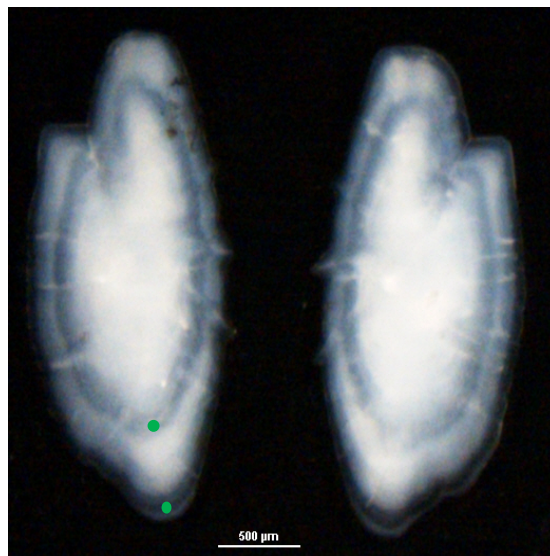
Date of capture: March 16, 2016.

Length: 165 mm.

Age determination: 2- or 3-years old?

Otolith Interpretation: Difficult: Rings marked on the rostrum and anti-rostrum. Of the 1st winter annual ring to the hyaline edge, there is one almost equidistant strong hyaline ring which might be a spawning check (C 18) or a true winter ring (then it would show an atypical growth pattern). The edge seems narrow hyaline (Hn)

Problems identification: Difficulties in distinguishing between C18 or A2 because of the strong hyaline mark.

- Otolith 3: 1st semester

Date of capture: May, 2016.

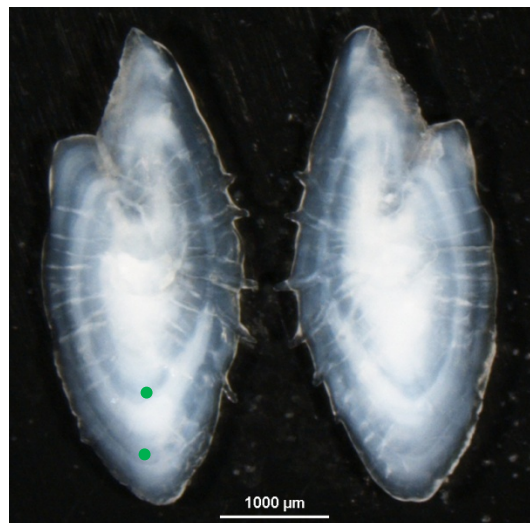
Length: 127 mm.

Age determination: 2 years old.

Otolith Interpretation: Two strong and well-marked winter hyaline rings + On. This corresponds to a typical otolith growth pattern.

Problems identification: The 1st winter annual ring and the opaque edge on the post-rostrum. The first inner winter ring (A1) has a radius measurement: $R1\ 745.82\ \mu\text{m}$, then if applying the Rule of the minimum R1 size it could be ascribe to a central check C08. Then this seems to be an Invalid application of the Radius R1 measurement, and a good example of the cases where visual interpretation should prevail over quantitative measurements. This could also be an otolith where ulterior analysis with micro-increments can serve to verify the distinction between age 1 and older fish (as we prefer to interpret in this case).

- Otolith 4: 2nd semester



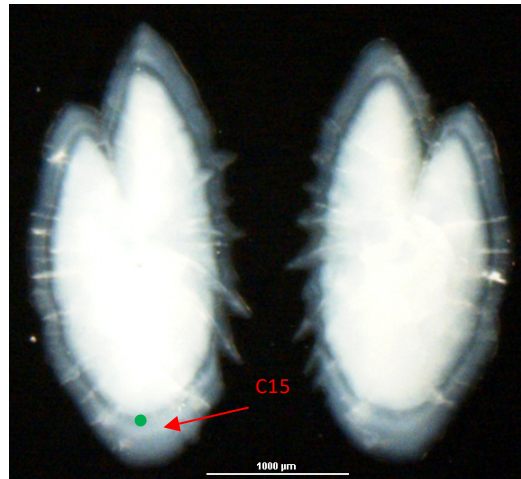
Date of capture: September, 2015.

Length: 153 mm.

Age determination: 2-years old.

Otolith Interpretation: The 1st winter annual ring with radius measurement: $R1\ 1071.41\ \mu\text{m}$ and the 2nd winter annual ring + wide opaque growth and a strong Hyaline edge.

Problems identification: Center ring would look like a check, but when measuring the radius is a real ring. In addition there are doubts whether this might be a three years old fish with a little opaque growth just at the edge of the otolith (implying little growth by September) (if this otolith would have appeared in June or July allocated age would have been 3 y.o.).

- Otolith 5: 2nd semester

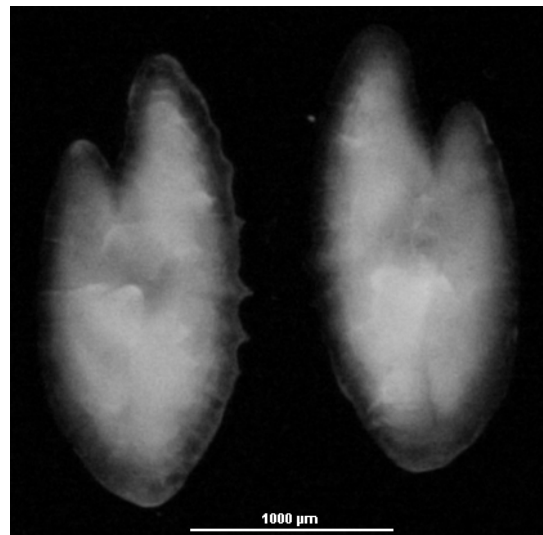
Date of capture: October, 2015.

Length: 156 mm.

Age determination: 1 year old.

Otolith Interpretation: Annual ring marked and appreciable throughout the structure of the otolith + wide opaque growth and a Hyaline edge

Problems identification: Ring weak and little defined parallel with the annual winter ring, spawning check C15

- Otolith 6: 2nd semester

Date of capture: November, 2016.

Length: 96 mm.

Age determination: 0 years old.

Otolith Interpretation: Annual winter ring forming on the edge of the otolith followed by opaque growth. So it has a hyaline mark before the edge (C08) then the edge becomes opaque again.

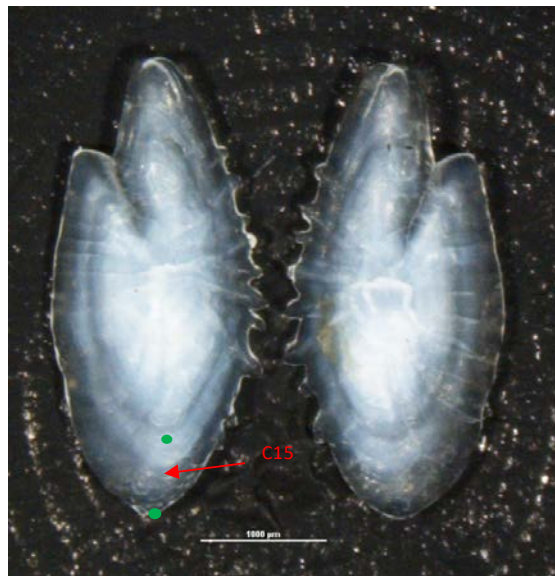
Problems identification In the second half of the year the hyaline edges are not taken into account for age assignation, because annual winter ring is being formed in the last quarter of the year. The opaque edge seems to suggest that the first winter zone might become a multiple mark winter zone if other marks would appear later in winter (as in January or February).

7.2.2 Division 9a

7.2.2.1 Subdivision 9a North

Conventional Birthdate: 1 January

- Otolith 1: 1st semester



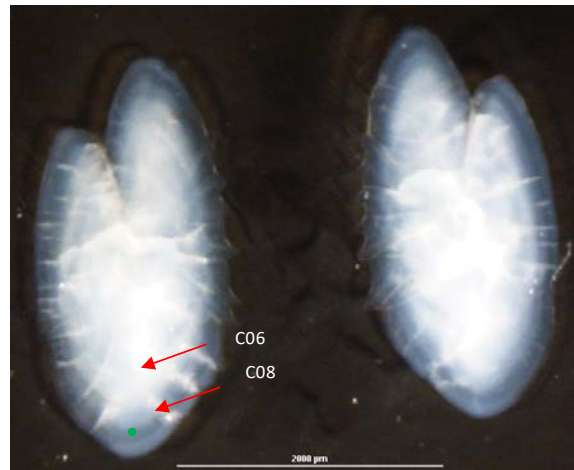
Date of capture: June, 2015.

Length: 142 mm.

Age determination: 2 years old.

Otolith Interpretation: The 1st winter annual ring with radius measurement: R1 1084.56 µm and the 2nd ring equidistant to the hyaline edge is a spawn check C15. The second winter ring is placed at the edge of the otolith (which is hyaline).

Problems identification The 2nd visible ring is not winter, there is little estimated growth until the next winter ring corresponding to the hyaline forming on the edge, so it is a spawning check C15

- Otolith 2: 1st semester

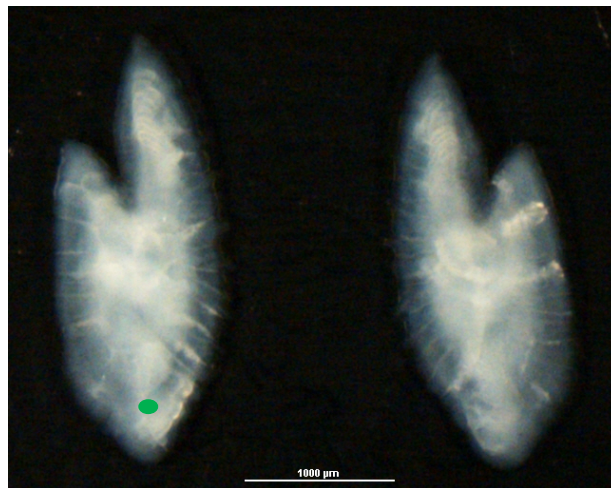
Date of capture: March, 2016.

Length: 108 mm.

Age determination: 1 year old.

Otolith Interpretation: The 1st winter annual ring with the opaque edge. Very weak central marks taken as check C08 and C06.

Problems identification Real winter rings are more strongly marked.

- Otolith 3: 2nd semester

Date of capture: November, 2016.

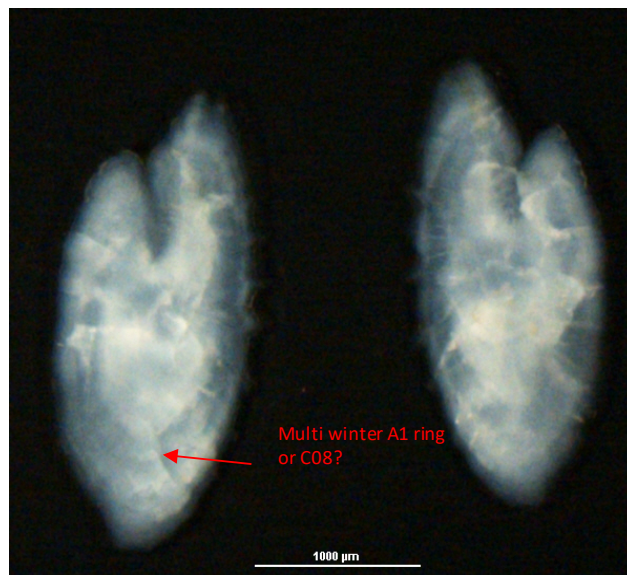
Length: 118 mm.

Age determination: 1 year old. (Doubts with Age 0)

Otolith Interpretation: The 1st winter ring has a radius measurement of 971.78 µm. Hyaline edge (but not much clear).

Problems identification Wide winter ring and not well marked around the otolith. There are doubts if this might be an Age 0 with just a check (C06). Measurement of the mark led to admit this as a winter ring. This would be a good candidate for analysis with microincrements to dilucidate between age 0 and age 1 individuals

- **Otolith 4: 2nd semester**



Date of capture: November, 2016.

Length: 124 mm.

Age determination: Doubtful between 0- or 1-year old.

Otolith Interpretation: The central ring with radius measurement: R1 697.98 μm it might correspond to a check C08 though it has an appearance of a true broad winter band as described by Aldanondo *et al.* (2016), so interpretation is doubtful. At the edge some hyaline edge formation seems to be occurring.

Problems identification Central ring mark can be identified as annual (winter) ring or as a check (by taking measurements of the radius it might be a false ring (size < mean C08 size of 780 μm in Hernandez *et al.* presentation 3), but this is not a sufficient argument); therefore actual interpretation and age allocation is doubtful. This could be a good candidate for micro-increment analysis (as Age 0 in November, would be accurately identified as Age 0 by this technique).

7.2.2.2 Subdivision 9a South (Gulf of Cadiz)

Conventional Birthdate: 1 January

7.2.2.2.1 Examples of typical otoliths in which growth patterns are of easy or sound interpretation

- **Otolith 1**. First semester, AGE 1



Date of capture: April 2015.

Length: 111 mm

Age determination: Age 1

Otolith Interpretation: The otolith shows a well-defined hyaline winter ring and a small growth compatible with the date of capture (April) plus some hyaline edge.

- **Otolith 2**. First semester, age 1. More growth



Date of capture: June 2009.

Length: 138 mm

Age determination: Age 1

Otolith Interpretation: In addition to the strongly marked hyaline zone, a wide opaque band corresponding to the season's growth is present.

- **Otolith 3.** First semester, age 2



Date of capture: April 2009. Length: 129 mm

Age determination: Age 2

Otolith Interpretation: The presence of several hyaline rings forming a complex compatible with an A1 ring and checks and the wide, hyaline edge, suggest an Age 2 anchovy without any growth in the season, as expected.

- **Otolith 4.** Second semester, Age 0



Date of capture: August 2015.

Length: 99 mm

Age determination: Age 0

Otolith Interpretation: Absence of any marks/rings, small size.

- **Otolith 5.** Second semester, age 0



Date of capture: July 2015.

Length: 79 mm

Age determination: Age 0

Otolith Interpretation: Very similar to the previous example, some small marks could be observed though they don't meet the conditions to be evaluated as winter rings.

- Otolith 6. Early Second semester, age 1



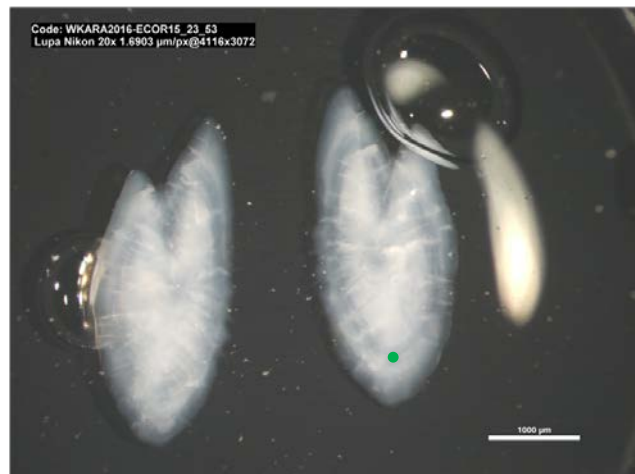
Date of capture: July 2015.

Length: 150 mm

Age determination: Age 1

Otolith Interpretation: The otolith shows the typical pattern for such age/season, with a strong marked first winter hyaline ring followed by an opaque band corresponding to the season's growth.

- **Otolith 7.** Late second semester, age 1



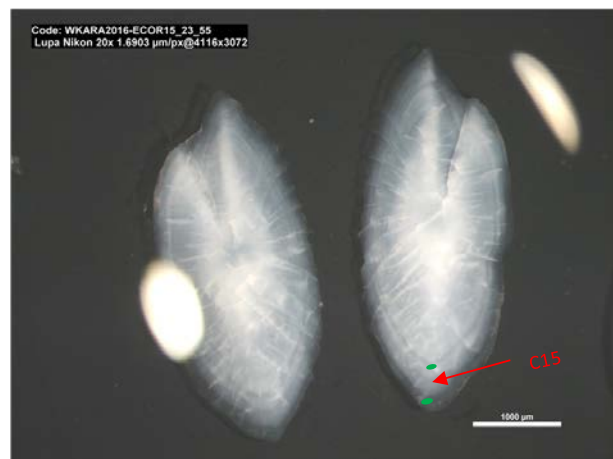
Date of capture: October 2015.

Length: 171 mm

Age determination: Age 1

Otolith Interpretation: The otolith shows a broader opaque band (the more time growing, the broader opaque band). In this case, no checks appear in the otolith; despite some spawning/summer checks could occur.

- **Otolith 8.** Second semester, age 2



Date of capture: October 2015.

Length: 174 mm

Age determination: Age 2

Otolith Interpretation: The otolith shows an internal hyaline zone followed by the first winter ring, in this case, very narrow but strongly marked all around the otolith. A small C15 check is observed as well (red arrow), to which the second winter hyaline ring follows and finally an opaque band corresponding to the most recent season growth (Ow).

7.2.2.2.2 Examples of typical otoliths with difficult interpretation

First an introduction to the difficulties found is summarized:

Reading otoliths from ICES Subdivision 9a-South is difficult and arises from several factors:

- **Incomplete temporal coverage** due to fishing regulation, which makes very difficult to analyse otoliths coming from at least (depending on the year) December and January.
- **Heterogeneous length classes coverage**: samples coming from commercial landings differ from those coming from surveys, and in the latter case, while the length coverage is greatly improved, their temporal coverage is narrower.
- **Differences in spatial coverage** between commercial landings sampling and survey sampling.

These constraints make difficult to build complete growth schemas which could help in age assignment: We know how the otoliths *are*, but for not well sampled length classes, we lack of clues about how they have been and how they progressed to its present state. This could be especially true for length classes which are not caught by the commercial fleet but studied in the surveys.

Moreover, there are other factors related to the biology of the species:

- Both biological sampling of adults and the presence of larvae and eggs of anchovy from March to November (Baldó *et al.*, 2006) suggest that anchovy in the Gulf of Cadiz shows a **protracted spawning season** which could cause that anchovies from **different spawning batches** (say Spring, Summer and Autumn-born for illustration purposes) could show **different growth patterns (Figure 7.2.2.2.1)**, providing that the most important events in their life (spawning, winter...) will happen at different *real* ages and probably at different sizes.
- Compared to spring/summer born anchovies, **an anchovy born close to the end of the spawning season** (late autumn) will reach winter at a minor size, thus laying the hyaline edge at a position closer to the nucleus. In the following year, providing it will probably have a *growth potential* greater than the spring/summer born anchovies due to its youth, the growth could be significantly different, even growing more in its second year of life than in the first.
- In the other hand, **an anchovy born in spring or summer** will reach winter at the same time, but having lived for up to 8 additional months (in the extreme case of March-born anchovy vs. November-born anchovy). It will reach winter at a larger size than autumn-born one, thus recording hyaline edge in a different position.

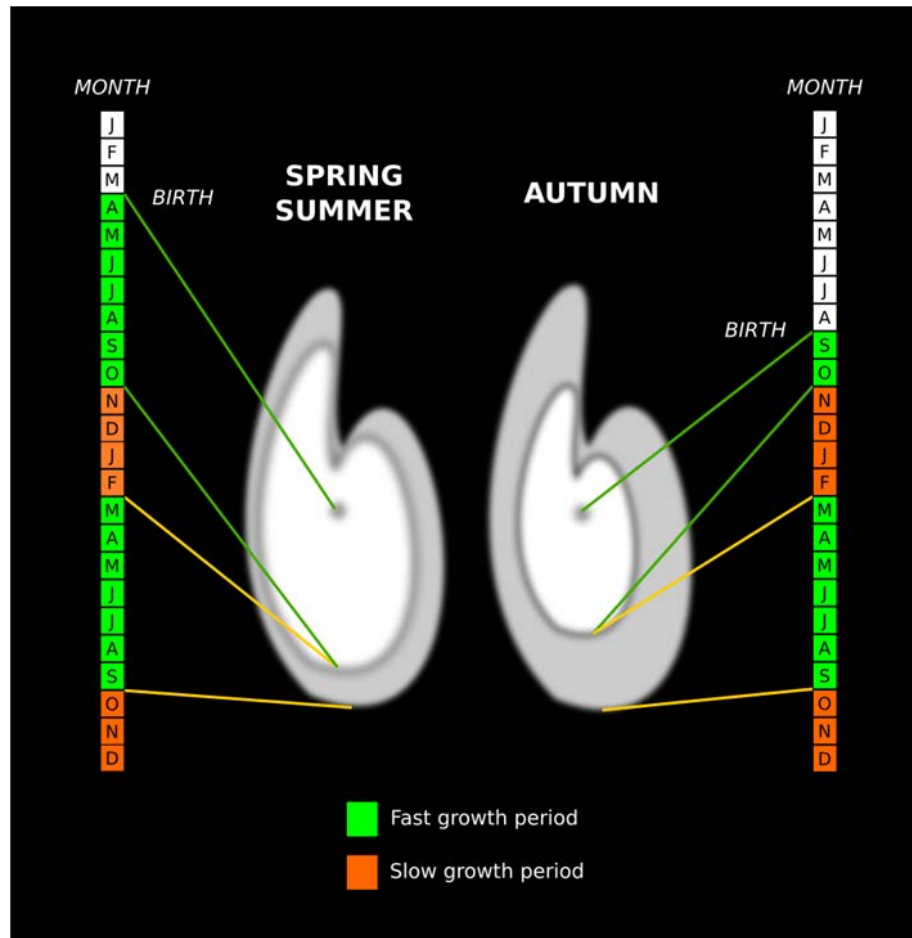


Figure 7.2.2.2.1. Interpretation of the effect of real birth date on growth pattern of 1-year old anchovies otoliths caught in Autumn.

- 1) **Presence of a growth pattern very different from expected:** too much/too few growth for the last or a particular season.
- 2) Alterations in the typical aspect of the otolith:
 - **The silent otoliths** show a high opacity/translucency which masks critical features for reading the age.
 - **The chatterbox otoliths**, which show a multi-ring, more or less evenly marked pattern in which many interpretations could fit.
- 3) Otoliths with extemporaneous features, like hyaline edges in summer or absence of previous marks (especially winter ring) in specimens caught in late winter/early spring.

It is worth to note that, mostly in the latter cases, **age reading to a particular otolith is not necessarily difficult**, but in their *surviving fellow anchovies* sharing such particular features, **the record of future real life events could be concealed in the otolith**. For instance, otoliths with absence of winter rings could be misclassified in future as younger specimens, and among those which laid hyaline material in summer, some could be misclassified as age group 1 even when other features of the specimen discourage such classification.

1. Extemporaneous opaque edge, first semester.



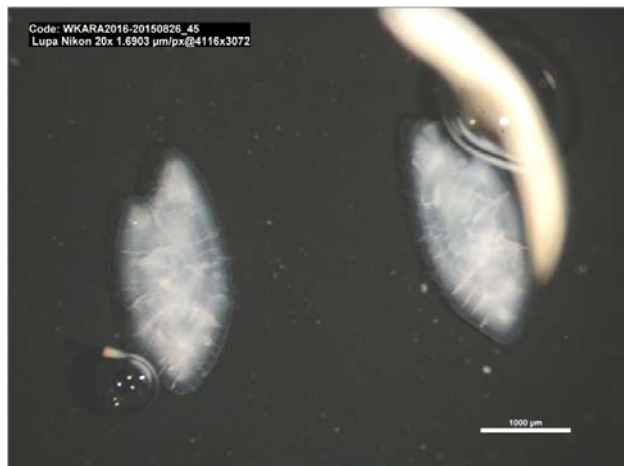
Date of capture: February, 2015

Length: 92 mm.

Age determination: Age 1

Otolith Interpretation: This otolith shows an opaque edge in late winter (February) and absence of winter rings. As stated before, there is no problem in age assignation for this kind of otoliths, but in anchovies with this growth pattern future misclassification could occur (in July for instance) due to the absence of first winter ring, thus underestimating the age.

2. Extemporaneous hyaline edge, second semester



Date of capture: August, 2015

Length: 100 mm

Age determination: Age 2?

Otolith Interpretation: This pattern could lead to misclassification in several ways, depending on how the particular pattern is interpreted. The presence or absence of checks and the size of the fish/otolith among other specific features may be taken into account for age assignation thus compromising the accuracy in age assignation.

Discussion: This and the following otolith examples seem 2 year-old fish just starting laying down its second opaque growth. The edge seems opaque (at least in the picture when amplified), although the colour is simply grey because of being the thin edge of the otolith. The actual second hyaline winter ring is left just before the opaque edge very close to it. This is August and the opaque edge of these otoliths is very small (On).

By taking into account the very small size of the individual it might be suspected they are just 1 y.o. with strong spawning check close to the edge (C15 or C18), but the position and growth till the mark before the edge seems to correspond to an entire second annual opaque growth for anchovies. Therefore interpretation of such mark as true winter ring has been preferred leading to allocate it as Age 2. The problem which puts uncertainty relates to the fact that for August, the opaque growth of the year would be too small, and that's not common at all for GoC anchovy otoliths. Therefore in case the second hyaline mark would actually be a check the preferred interpretation put forward before would lead to age overestimation.



Date of capture: August, 2015

Length: 105 mm

Age determination: Age 2?

Otolith Interpretation: Same case as above.

Suggestion: These two otoliths are good candidates for micro-increment analysis to elucidate whether they are just 1 y.o. (with a strong spawning check) or actually a 2 year old fish.

3. Silent multi-ring otolith



Date of capture: April, 2015

Length: 109 mm.

Age determination: Age 1???

Otolith Interpretation: A mixture of multi-ring otolith, in which many non-preponderant rings and a general faint aspect make age assignation based on recognizable patterns very difficult. Most feasible interpretation could be 1 y.o. in April just having led down a multiple (triple?) weak winter ring

4. Talkative, multi-ring otolith



Date of capture: May, 2015

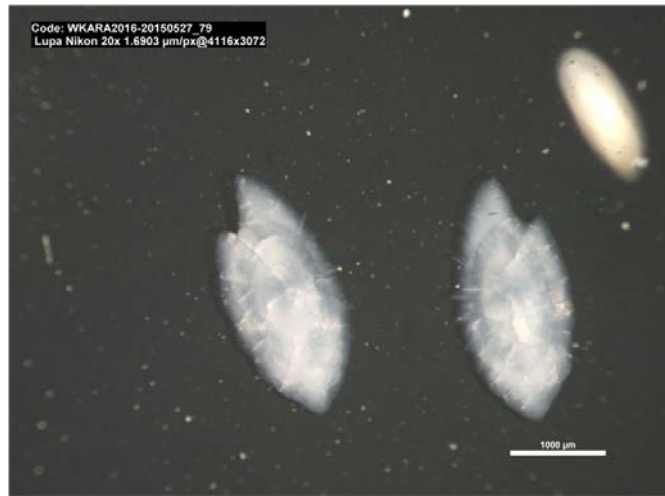
Length: 127 mm.

Age determination: Age 1-4?

Otolith Interpretation: These otoliths show many well-marked rings which could lead to several age interpretations. In this case, the two most external hyaline rings make age estimation difficult. The outermost opaque band seems to be too narrow for the season's growth for an age 1 specimen, but at the same time, interpreting A1 as the second hyaline rings leads to a huge growth plus one check. Considering the latter as A1 and the outermost hyaline ring as A2 implies an excessive growth for an age 2 anchovy, taking into account that its growth starts later in the season. Therefore it seems that we see a 1 y.o. with C05 and C15 (the latter laid down earlier during Spring). Another way of looking at this is that the range between the last two rings is the grown from late autumn to late

February-March of the year being caught showing a recent opaque growth On till the opaque edge.

5. Much growth, inner ring



Date of capture: May, 2015

Length: 105 mm.

Age determination: Age 1-2???

Otolith Interpretation: Very difficult: There is a multiple weak inner ring assumed to correspond to the first winter ring. The edge seems entirely opaque so it all seems to be a very wide growth of the year (in May!!!). So this would be 1 y.o., but in case it would lay down afterwards a spawning check, it could be confused during the second half of the year with a 2 y.o.! (See cases of 2. Extemporaneous hyaline edge, second semester otoliths above). The alternative would be assuming or believing that a second winter ring would be appearing at the edge and then allocating the fish to Age 2 (though the hyalinity at the edge is very limited to the antirrostrum of the right image).

6. Late first semester or very early second semester with little opaque deposition.



Date of capture: Late June, 2015

Length: 113 mm.

Age determination: Age 1-??

Otolith Interpretation: This otolith shows typical multiple inner A1 ring plus a seasonal increment (the opaque band till the edge), but it looks narrower than expected in late June. Taking into account that similar or even greater growths are observed in April or even late March. This type of otoliths alters a bit the pattern observed during the year.

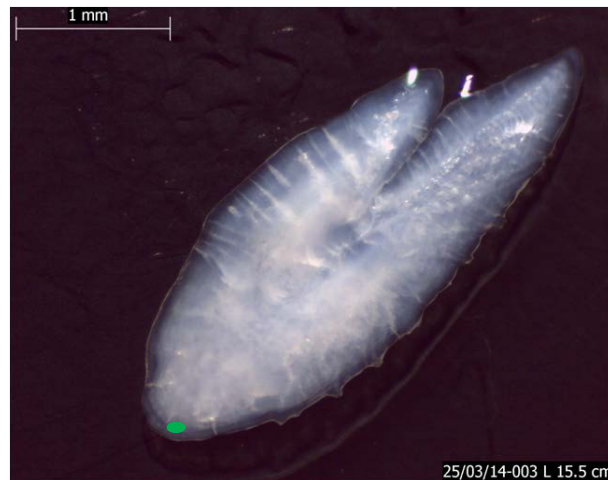
7.2.3 Alboran Sea

Conventional Birthdate: 1 July

1st semester:

All Otoliths seem to correspond to age 0 after its first winter (for a birthdate in 1st July)

- Otolith 1:



Date of capture: March 2014

Length: 155 mm

Age determination: Age 0?

Otolith Interpretation: Age 0; not a very well-marked winter ring very close to the edge.

- Otolith 2:



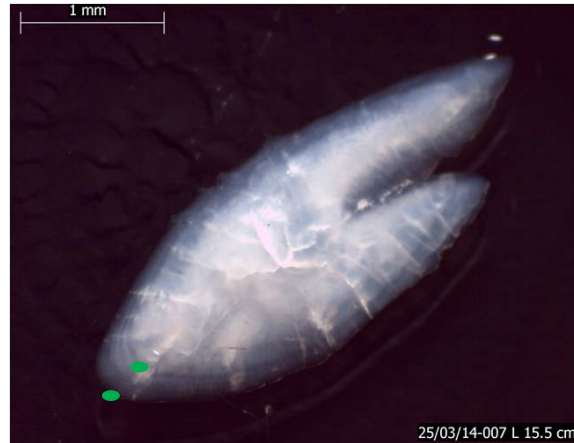
Date of capture: March 2014

Length: 155 mm

Age determination: Age 0-?

Otolith Interpretation: Age 0; not a very well marked winter ring.

- Otolith 3:



Date of capture: March 2014

Length: 155 mm

Age determination: Age 1? (But doubtful with age 0?)

Otolith Interpretation: Age 1; first winter ring quite clear and then the growth of the second year including a couple of checks. Doubts arose with Age 0. By comparison with the following example the growth from A1 to the Edge could have been laid down in the year of capture during winter and early spring. Unfortunately this is a rather typical otolith in the area. Good candidate for micro increment analysis to discriminate between Age0 and Age 1.

- Otolith 4:



Date of capture: May 2013

Length: 150 mm

Age determination: Age 0

Otolith Interpretation: Age 0; first winter ring quite clear and then the growth of the year including a couple of checks.

- Otolith 5:



Date of capture: May 2013

Length: 140 mm

Age determination: Age 0

Otolith Interpretation: Age 0; not a very well-marked winter ring.

- Otolith 6:



Date of capture: May 2013

Length: 150 mm

Age determination: Age 0

Otolith Interpretation: Age 0; first winter ring quite clear and then the growth of the year.

- Otolith 7:



Date of capture: May 2013

Length: 140 mm

Age determination: Age 0

Otolith Interpretation: Age 0; first winter ring quite clear and then the growth of the year.

Alboran Sea (GSA01) 2nd semester

- Otolith 8:



Date of capture: August 2013

Length: 150 mm

Age determination: Age 1

Otolith Interpretation: Age 1; first winter ring quite clear and then the growth of the year including a spawning check.

- Otolith 9:



Date of capture: August 2013

Length: 115 mm

Age determination: Age 0

Otolith Interpretation: Age 0; no winter ring at all. (Doubt with age 1. Good otolith for analysis with Daily Increments and clarify whether it is age 0 or age 1).

- Otolith 10:



Date of capture: August 2013

Length: 145 mm

Age determination: Age 1

Otolith Interpretation: Age 1; first winter a bit faint and then the growth of the year. (Doubt with age 0. Good otolith for analysis with Daily Increments and clarify whether it is age 0 or age 1).

- Otolith 11:



Date of capture: August 2013

Length: 120 mm

Age determination: Age 1 ??

Otolith Interpretation: Age 1; first winter ring a bit faint and then the growth of the year. (Doubt with age 0. Good otolith for analysis with Daily Increments and clarify whether it is age 0 or age 1).

- Otolith 12:



Date of capture: August 2013

Length: 105 mm

Age determination: Age 0

Otolith Interpretation: Age 0; no winter ring at all.

- Otolith 13:



Date of capture: August 2013

Length: 160 mm

Age determination: Age 2 (Age 1?)

Otolith Interpretation: Age 2; not very clear the first winter ring and then the second is quite close to the edge so it might be a spawning check, but it is more likely a two years old. Doubtful if all are checks (including spawning check) during its second year of life (Age1)

7.2.4 Western Mediterranean

Conventional Birthdate: 1 July

Northwestern Spain (GSA06) 1st semester

- Otolith 1:



Date of capture: June 2013 **Length:** 145 mm

Age determination: Age 0

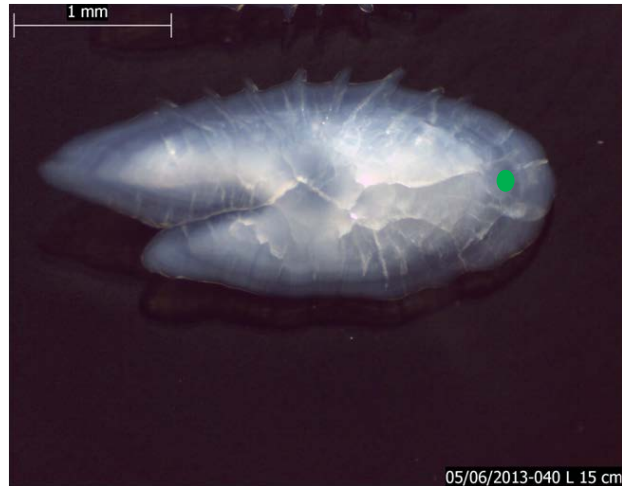
Otolith Interpretation: Age 0; first winter ring well marked and then the growth of the year.

- Otolith 2:

Date of capture: June 2013 **Length:** 150 mm

Age determination: Age 0

Otolith Interpretation: Age 0; winter ring well marked and then the growth of the year.



- Otolith 3:



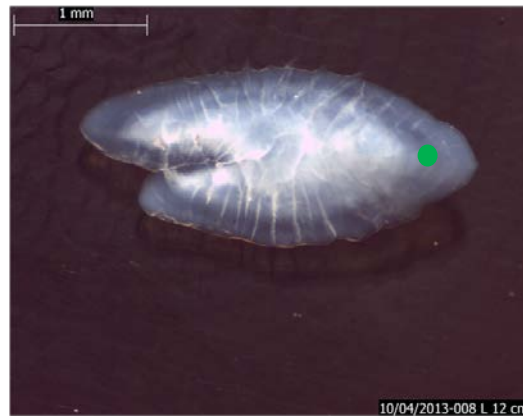
Date of capture: April 2013

Length: 110 mm

Age determination: Age 0

Otolith Interpretation: Age 0; winter ring well marked and then the growth of the year.

- Otolith 4:



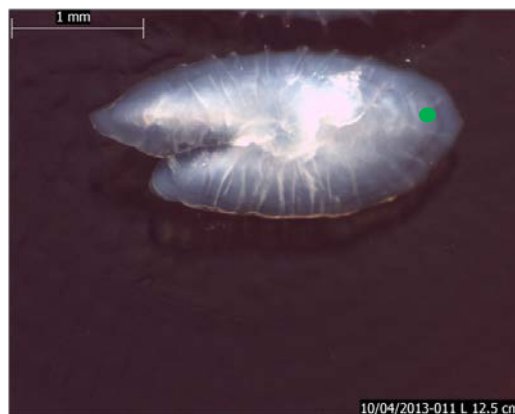
Date of capture: April 2013

Length: 120 mm

Age determination: Age 0

Otolith Interpretation: Age 0; winter ring well marked and then the growth of the year (Ow).

- Otolith 5:



Date of capture: April 2013

Length: 125 mm

Age determination: Age 0

Otolith Interpretation: Age 0; winter ring well marked and then the growth of the year.

- Otolith 6:



Date of capture: April 2013

Length: 135 mm

Age determination: Age 0

Otolith Interpretation: winter ring well marked and then the growth of the year.

- Otolith 7:



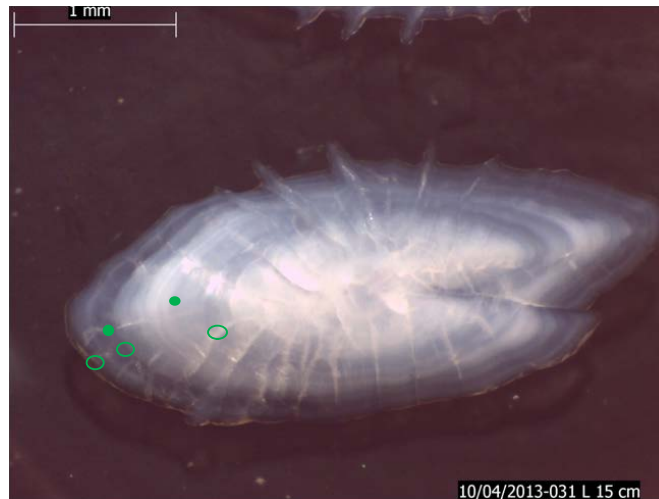
Date of capture: April 2013

Length: 140 mm

Age determination: Age 0?

Otolith Interpretation: Age 0; first check C06, then a winter ring well marked and then the growth of the year. Very doubtful it could also have two winter rings being Age 1 (A1+A2+Ow), though OW in April would be very unlikely in the Bay of Biscay (the opaque edge in April is more typical of Age 1 than of Age 2 in the Bay of Biscay). This could be a candidate for micro-increment analysis to see if this Age 1 just after its first winter or an older one. Measuring the R1 could also give a clue to decide provisionally on an age.

- Otolith 8:



Date of capture: April 2013

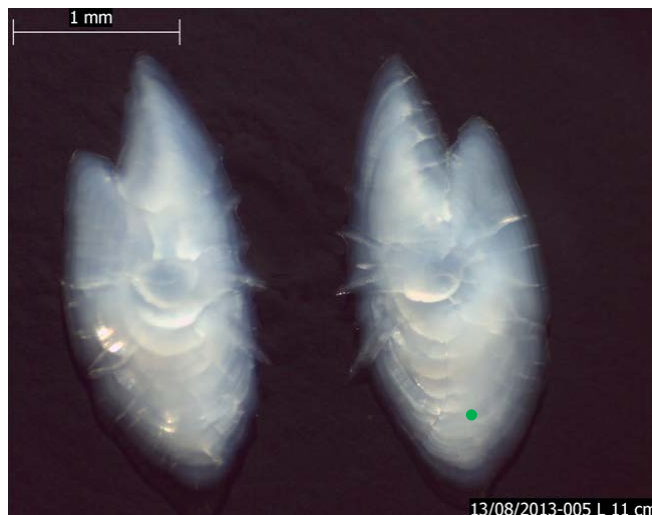
Length: 150 mm

Age determination: Age 1? or Age 2?

Otolith Interpretation: Interpretation Age 1 (full green points); first check C06, a first winter ring (A1 inner green point, a bit faint), the second one (A2, second green point well marked) and then the growth of the year. Interpretation Age 2 (open green circles): A1, A2, A3 (A3 corresponding to the Hyaline edge). The growth between A2 and the edge is typically complete expected growth at Age 2 in the Bay of Biscay. This together with the observation of the hyaline edge leads to the second interpretation (Age 2), because the third winter ring at the edge is typical of old fish in April without resuming the opaque growth (as in the Bay of Biscay).

Northwestern Spain (GSA06)) 2nd semester

- Otolith 9:



Date of capture: August 2013

Length: 110 mm

Age determination: Age 1

Otolith Interpretation: Age 1; winter ring a bit faint and then the growth of the year. Doubtfull because the most intense marked ring is quite close to the edge. If that were the winter ring then the opaque growth of the year for an Age 1 would have been too little (so it might be just a spawning check). Another inner mark is proposed for A1 (full green point) but it is very weak and it can only be seen clearly in the rostrum (but it could just be a check).

- Otolith 10:



Date of capture: August 2013

Length: 115 mm

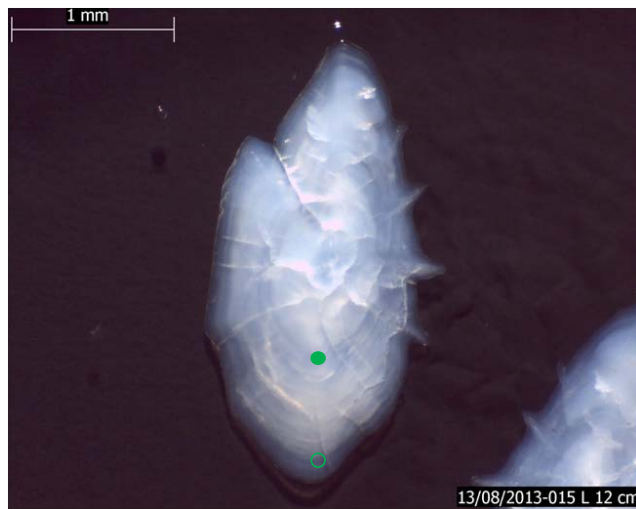
Age determination: Age 1?

Otolith Interpretation: Two potential interpretations: a) hyaline winter ring a bit faint close to the edge (open green circle) and then the growth of the year On (but rather small for August) or b) Winter ring being the first inner marks (full green point) followed by a wide opaque growth of the year till August showing a spawning check close the edge (open green circle).

Another Doubt comes from: if the first and second marks would be true winter rings (the second followed by On) then this would be Age 2.

Good candidate for microanalysis to elucidate if this is just a 1 y.o. and which are winter rings

- Otolith 11:



Date of capture: August 2013

Length: 120 mm

Age determination: Age 1??

Otolith Interpretation: similar interpretations and doubts as the former otolith

- Otolith 12:



Date of capture: August 2013

Length: 125 mm

Age determination: Age 1

Otolith Interpretation: Age 1; first check C04, then a winter ring and then the growth of the year.

- Otolith 13:



Date of capture: August 2013

Length: 130 mm

Age determination: Age 1

Otolith Interpretation: Age 1; winter ring well marked and then the growth of the year and a spawning check.

- Otolith 14:



Date of capture: August 2013

Length: 145 mm

Age determination: Age 1

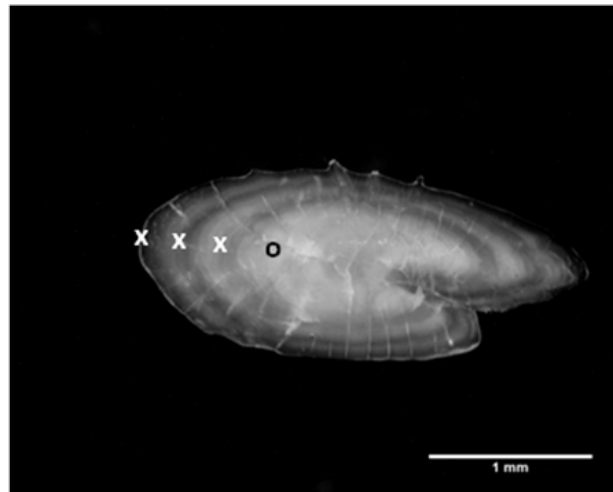
Otolith Interpretation: Age 1; winter ring well marked and then the growth of the year and a spawning check.

7.2.5 Tyrrhenian Sea

Conventional Birthdate: 1 July

Taking into account the otolith sample from this area (Central Southern Tyrrhenian Sea) they have been chosen 3 otolith as examples of clear reading, and 3 difficult reading. In this way we indicate the main difficulties in the ageing analysis. In the images: the winter rings are indicated with a "X"; the false ring is indicate with "o".

- Otolith 1:



Date of capture: 08/03/2013

Length: 130 mm

Sex: female

Age determination: Age 2

Otolith Interpretation: the otolith shows three clear winter ring (one on the edge) that they can be followed around all otolith with a distance between the rings almost not decreasing with the age.

Doubts for the picture: Given the very intense and similar aspect of the hyaline marks they all seem true winter rings and therefore it is admitted to be Age 2 (with the third ring at the edge, taking into account the date of capture and 1 July as the birthday). However the resulting growth pattern shows very similar growth bands between successive Ages, which does not match with the usual growth pattern in other areas. Moreover before the first winter ring it is possible see a false ring, indeed it is not possible follow it on all otolith and it is not deep marked as the winter rings.

- Otolith 2:



Date of capture: 08/03/2013

Length: 115 mm

Sex: female

Age determination: Age 1

Otolith Interpretation: The otolith in this picture present two winter (one on the edge) and based on the date of capture and birthday it has an age of one

- Otolith 3:



Date of capture: 10/09/2013

Length: 95 mm

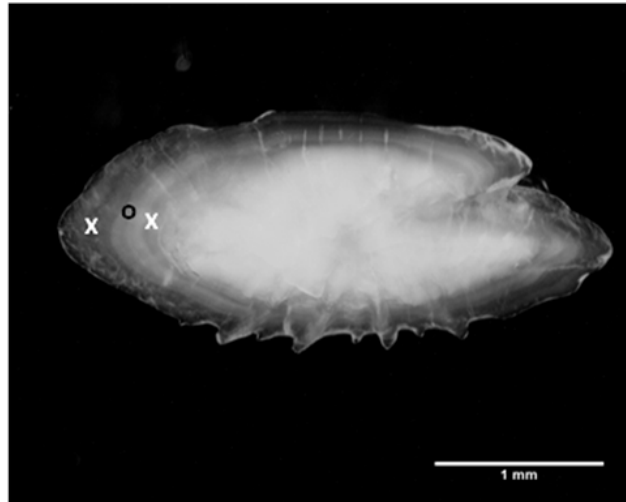
Sex: male

Age determination: Age 1

Otolith Interpretation: This picture show an otolith with an opaque edge and just one winter ring with an age of 1 year

These first otoliths suggest that the growth pattern in this area is different from the remaining areas examined in the workshop as far as after age 0 the other sub-sequent growths seems to be very similar one each other (but see final comments below).

- Otolith 4:



Date of capture: 08/09/2013

Length: 130 mm

Sex: female

Age determination: Age 2

Otolith Interpretation: In this picture it is showed the presence of reproductive false ring (C15) between the first and second winter ring

- Otolith 5:



Date of capture: 18/11/2013

Length: 135 mm

Sex: female

Age determination: Age 1

Otolith Interpretation: The winter rings are indicated with a “X”; the false ring is indicated with “o”. In the following picture a false ring is indicated before the first winter ring (C06). The second winter ring is very well marked but just being formed at the edge (so it is the one expected to correspond to the next coming winter), so it is not counted to assign age during the second half of the year (thus Age= 1).

- Otolith 6:



Date of capture: 18/09/2013

Length: 145 mm

Sex: female

Age determination: Age 2

Otolith Interpretation: In the picture it is showed that in some cases the best portion to read the otolith is the anterior part, given that marks are very weakly marked in most of the otolith.

The growth pattern depicted in these three last figures (particularly in the otolith 4) is more similar to the other areas examined in the workshop, for progressive decreasing of growth bands between subsequent age classes.

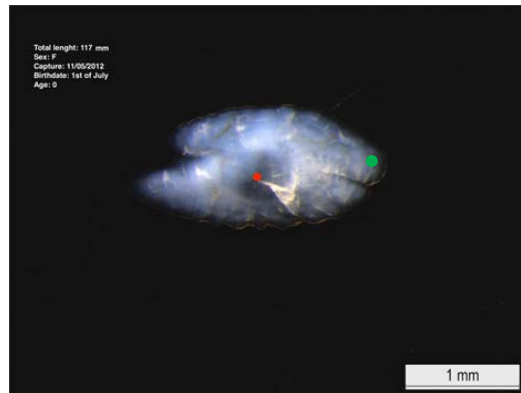
7.2.6 Strait of Sicily

Conventional Birthdate 1 July

7.2.6.1 Examples of typical Otoliths in which growth pattern are of easy or sound interpretation.

1st semester

- Otolith 1:



Date of capture: 11/05/2012

Length: 117 mm **Sex:** female (maturity stage 3)

Age determination: Age 0

Otolith Interpretation: If the hyaline edge is considered to correspond to the first winter ring (A1) then based on capture date and conventional birthdate the fish is of Age 0 (Age=WR-1). The problem is that in such a case no opaque growth of the year would have occurred till May. The Alternative interpretation would be that this fish is born in the current year during winter (age -1?). If these otoliths are common then moving the birthdate to January would help in solving coherent naming and grouping of year classes throughout the year. This is a good candidate for micro-increment analysis to clarify when these fish were born.

- Otolith 2:



Date of capture: 11/05/2012

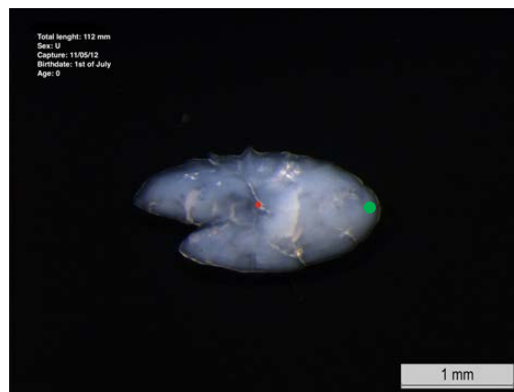
Length: 118 mm

Sex: male

Age determination: Age 0

Otolith interpretation: One marked hyaline ring is observed; based on capture date and conventional birthdate the fish is of Age 0

- Otolith 3:



Date of capture: 11/05/2012

Length: 112 mm

Sex: indeterminate

Age determination: Age 0

Otolith interpretation: If the hyaline edge is considered to correspond to the first winter ring (A1) then based on capture date and conventional birthdate the fish is of Age 0 (Age=WR-1).

The Alternative interpretation would be that this fish is born in the current year during winter (age -1?) If these otoliths are common then moving the birthdate to January would help in solving coherent naming and grouping of year classes throughout the year. This is a good candidate for micro-increment analysis to clarify when these fish were born.

- Otolith 4:



Date of capture: 11/05/2012

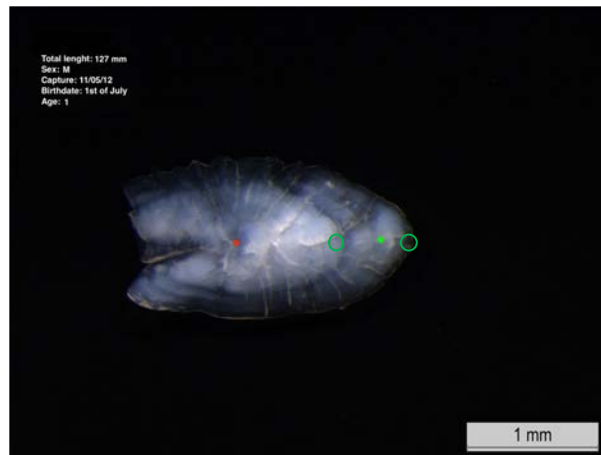
Length: 129 mm

Sex: female

Age determination: 0

Otolith interpretation: green point= winter ring. Based on capture date and conventional birthdate the fish is of Age 0.

- Otolith 5:



Date of capture: 11/05/2012

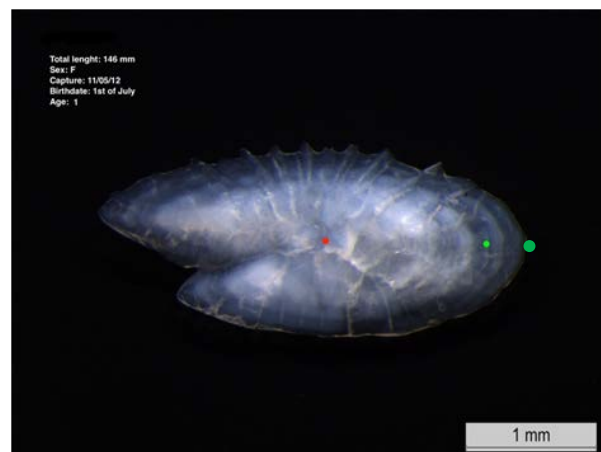
Length: 127 mm

Sex: male

Age determination: Age 1

Otolith interpretation: Interpretation Age 1: Two winter rings (open green circles) the second one at the hyaline edge (with C15 in the middle) (thus Age=WR-1=1). Interpretation as Age 0; multiple winter ring (the central one pointed with a full green point) (though the separations between the multiple marks are quite large, greater than normal); (Thus Age=WR-1=0)

- Otolith 6:



Date of capture: 11/05/2012

Length: 146 mm

Sex: female

Age determination: Age 1

Otolith interpretation: green point: 2 winter rings the second one just at the edge (hyaline edge). Based on capture date and conventional birthdate the fish is of Age 1 (Age =WR-1=2-1=1). Doubt if the first winter ring is just a multiple ring then we would have just a single winter ring + opaque edge (On) till the edge. Then Age=1-1=0.

2nd semester
- Otolith 7:



Date of capture: 30/08/2012

Length: 107 mm

Sex: indeterminate

Age determination: Age 0

Otolith interpretation: No winter ring therefore based on capture date and conventional birthdate the fish is of Age 0.

- Otolith 8:



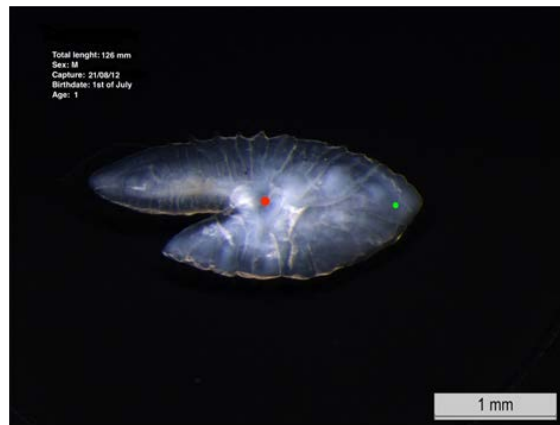
Date of capture: 30/08/2012

Length: 106 mm

Sex: indeterminate

Age determination: Age 0

Otolith interpretation: No winter ring, so based on capture date and conventional birthdate the fish is of Age 0.

- Otolith 9:

Date of capture: 21/08/2012

Length: 126 mm

Sex: male

Age determination: Age 1

Otolith interpretation: green point: One weak winter ring. Based on capture date and conventional birthdate the fish is of Age 1

- Otolith 10:

Date of capture: 21/08/2012

Length: 116 mm

Sex: female

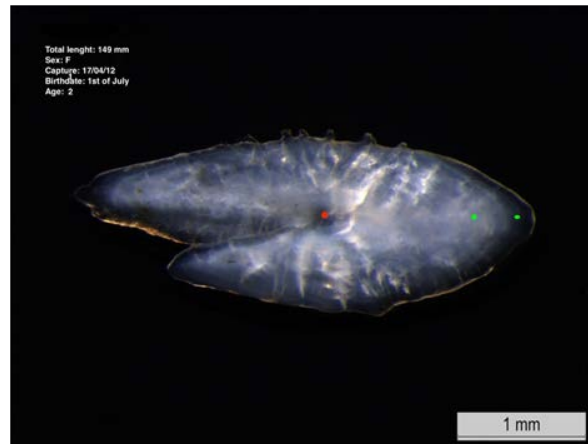
Age determination: Age 1

Otolith interpretation: green point: One winter ring; magnification: 25X. Based on capture date and conventional birthdate the fish is of Age 1.

7.2.6.2 Examples of typical Otoliths in which growth pattern are of hard interpretation.

1st semester

- Otolith 11



Date of capture: 17/04/2012

Length: 149 mm

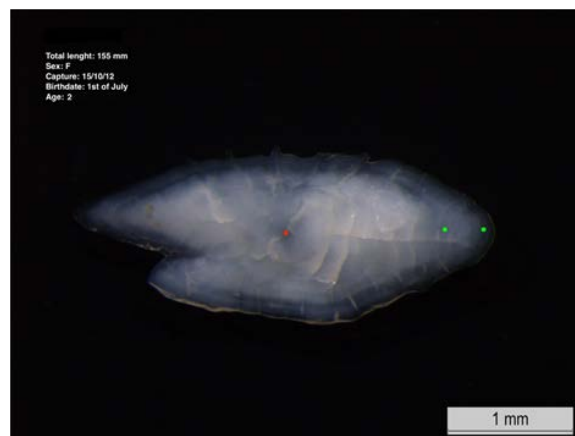
Sex: female

Age determination: Age 1

Otolith interpretation: green points: winter rings. Two winter rings (the second one at the hyaline edge) then based on capture date and conventional birthdate the fish is of Age 1 ($\text{Age} = \text{WR} - 1 = 2 - 1 = 1$). Alternative doubtful interpretation Age 0 if the first winter ring is just at the edge and the former mark is just a check (C08).

2nd semester

- Otolith 12



Date of capture: 15/10/2012

Length: 155 mm

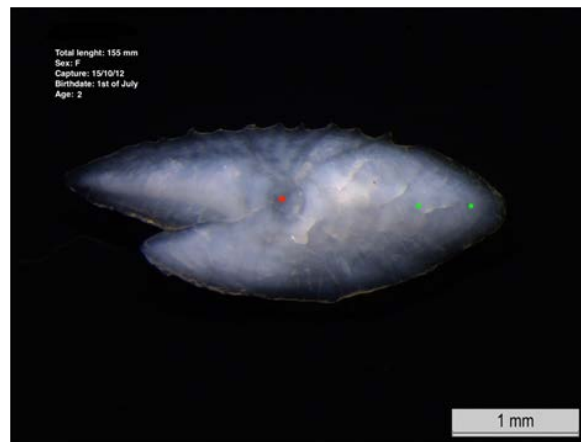
Sex: female

Age determination: Age 2

Otolith interpretation: green points: Two winter rings; magnification: 25X. Then Based on capture date and conventional birthdate the fish is of Age 2 ($\text{Age} = \text{WR} = 2$). Alternative doubtful interpretation for a single winter ring (the first one marked with green points)

whiles the second mark close to the edge would be a summer check. IN this case Age = WR=1

- Otolith 13



Date of capture: 15/10/2012

Length: 155 mm

Sex: female

Age determination: Age 2

Otolith interpretation: green points: winter rings. Based on capture date and conventional birthdate the fish is of Age 2. Doubtful: Alternative interpretations: No clear winter rings; other than at the edge, then either age 0 or age 1 is on winter ring is marked. The females was at maturity stage 5 (post-spawning) this indicated that it was not an age 0. Therefore this should either age 1 or age 2. Then this individual could be discarded from age reading. Good candidate for micro increments analysis to definitively discard Age 0 and to see if it can be identified as Age 1.

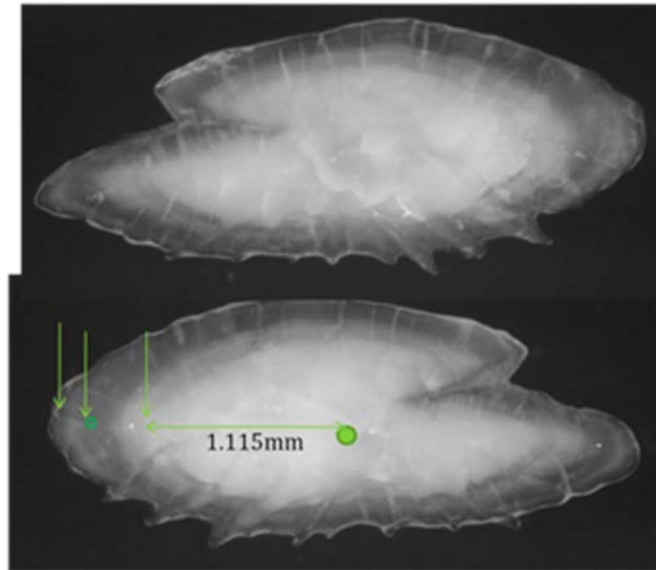
7.2.7 Eastern Ionian Sea

Conventional Birthdate: 1 July

It has been clarified that growth patterns may explain well otolith structures and they can be a significant interpretation tool although they cannot easily explain all cases. The explanation of growth patterns was suggested as a primary goal for each reader. It is preferred to spend time on matching growth patterns with the presence/absence of translucent zones rather than “searching” for hyaline rings only. The identification of the first annual ring (Otolith picture 1) and the edge type (Otolith pictures 1 and 2) is not always an easy problem to deal with and the wide spawning season may create increments at a variety of distances from otolith nucleus among individuals. Misinterpretations of WKARA (2009) age reading protocol (paragraph 9.9.2) were clarified: the otolith 2 was finally 1-year-old because it has two winter hyaline rings (the second one just at the edge), then applying the rule for a birthdate at 1st July for an anchovy caught in June, Age would equal the number of winter rings minus one, i.e. 2-1= 1 y.o. .

Finally, indirect validation methods and direct ones such as the previous work on otolith microstructure of North Aegean Sea anchovy larvae (Schismenou *et al.*, 2013) may help in the identification of the first annual ring and is probably a step forward on improving our techniques.

- **Otolith 1:**

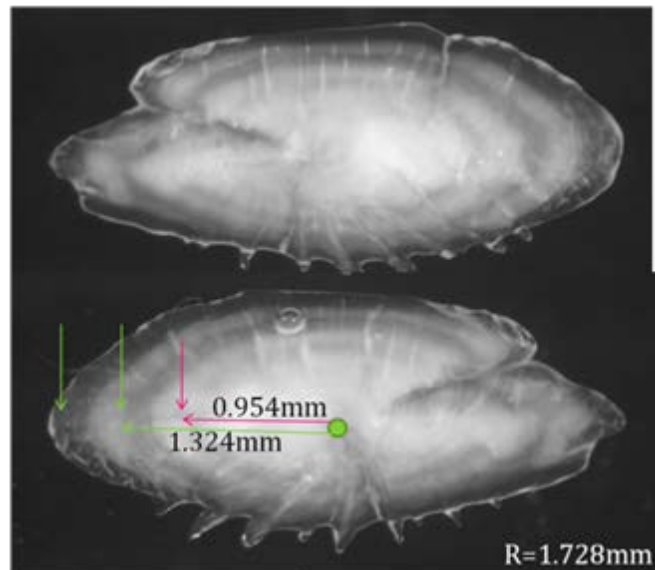


Date of capture: June 2014

Length: 147 mm

Age determination: Age 0

Otolith Interpretation: Green arrows show three hyaline rings. In this case the edge interpretation is difficult because it can be considered either as hyaline or as opaque ring. Furthermore the hyaline ring at 1.115 mm (first green arrow) is very thin and it is not easy to say that this ring is a hyaline winter ring. Therefore the first winter ring is probably the second arrow and the age is 0, or alternatively we have two winter rings (the second one at the edge) and the age is 1. The alternative of assuming the last hyaline edge as a true winter ring would lead to deduce age 1 to this fish (although the growth pattern would show a very limited growth in the previous year, at age 1, and a very weak hyaline mark for the second winter). Age 0 seems to be most likely interpretation of this otolith (with a single winter ring with the green circle plus the growth of the year and a potential hyaline check at the edge).

- Otolith 2:

Date of capture: June 2014

Length: 128 mm

Age determination: Age 1

Otolith Interpretation: Red arrow shows a rather false ring at 0.954mm from nucleus and green arrows show two hyaline rings.

7.2.8 Adriatic Sea

Conventional Birthdate 1 June

All of otoliths that were presented came from Medias survey. That would mean that all of them are from II semester because Medias survey in Croatia is carried out mainly in September, with some samples obtained from late August and early October. Spawning season lasts from April till October with spawning peak in a period from April till July. The 1st of June was used as a conventional birthdate for anchovy in Adriatic Sea. Typical lengths observed in the last four Medias surveys ranged from 6 cm to 17 cm with typical age class range from 0 to 2, in some cases also 3.

Red lines shows otolith radius, green one R1 radius and yellow one R2 radius.

- Otolith 1:

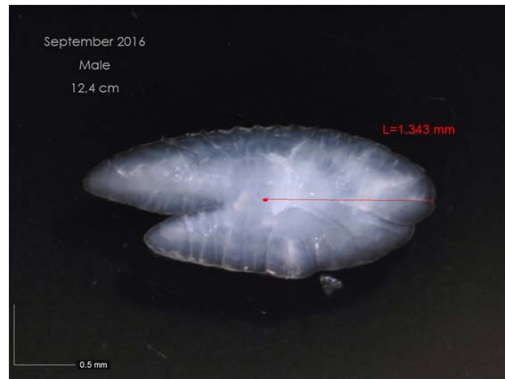
Date of capture: September 2016

Length: 103 mm

Age determination: Age 0

Otolith Interpretation: No visible rings, Age group 0

- Otolith 2:



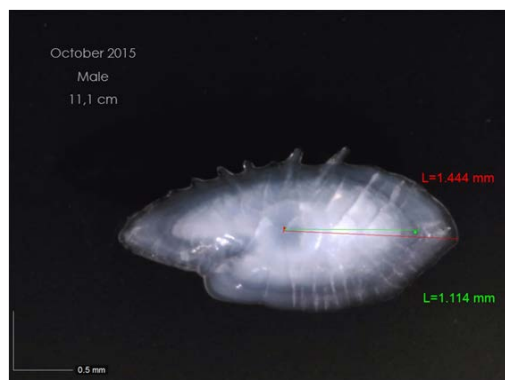
Date of capture: September 201

Length: 124 mm

Age determination: Age 0

Otolith Interpretation: No visible rings, Age group 0

- Otolith 3:



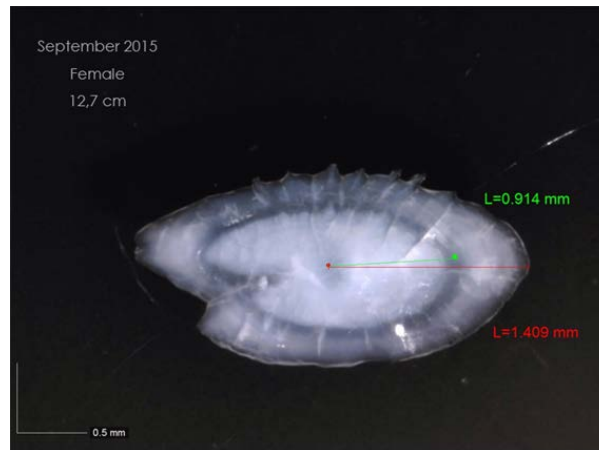
Date of capture: October 2015

Length: 111 mm

Age determination: Age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1. There is always doubt that R1 could be check.

- Otolith 4:



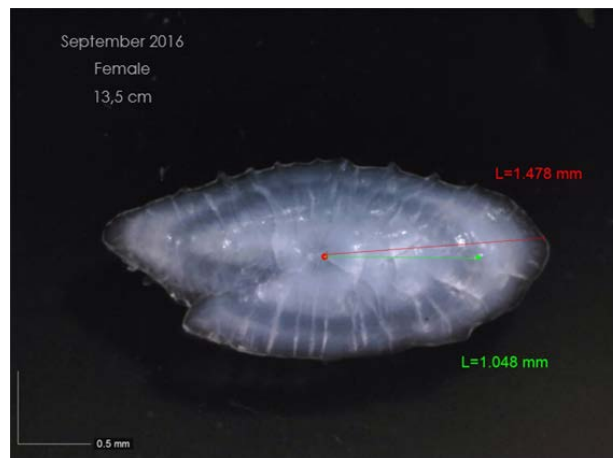
Date of capture: September 2015

Length: 127 mm

Age determination: Age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1. There is always doubt that R1 could be checked, especially in case of measurement of only 0,914 mm (but such criteria should not prevail over the visual interpretation of such clear marked ring being a winter ring).

- Otolith 5:



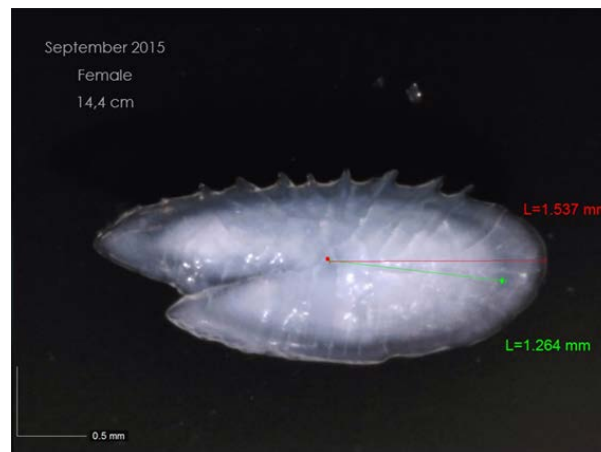
Date of capture: September 2016

Length: 135 mm

Age determination: Age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1.

- Otolith 6:



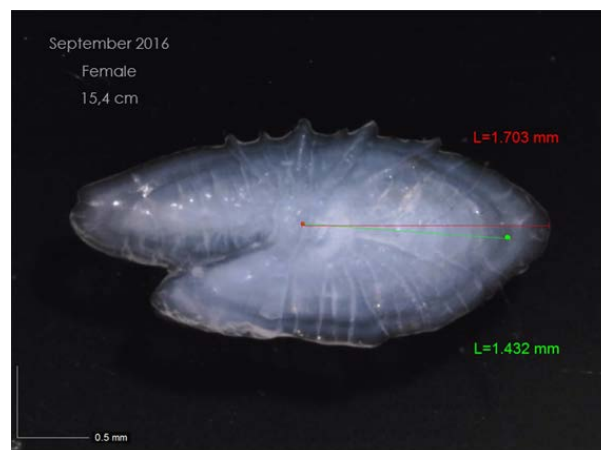
Date of capture: September 2016

Length: 144 mm

Age determination: Age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1.

- Otolith 7:



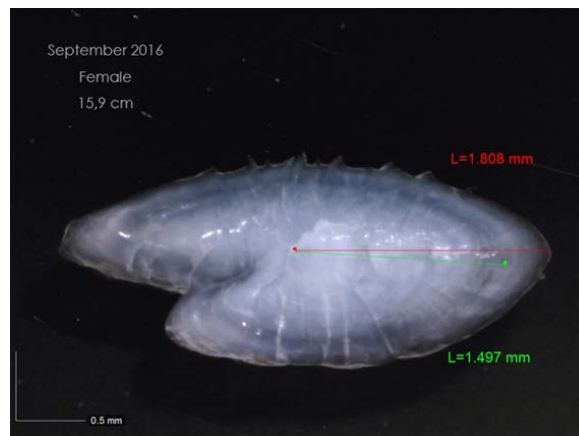
Date of capture: September 2016

Length: 154 mm

Age determination: age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1.

- Otolith 8:



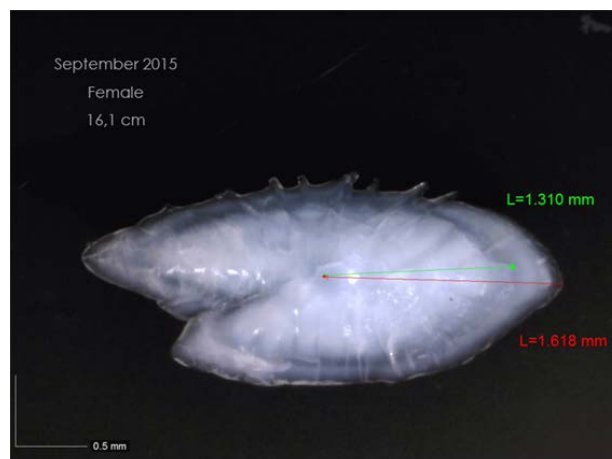
Date of capture: September 2016

Length: 159 mm

Age determination: age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1.

- Otolith 9:



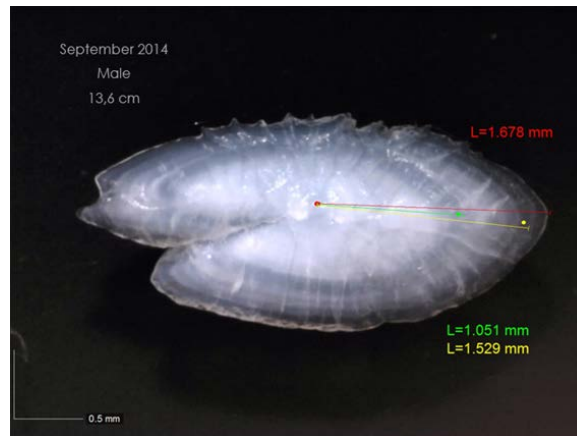
Date of capture: September 2015

Length: 161 mm

Age determination: age 1

Otolith Interpretation: Green measurement represents growth in first year of life; the interpretation would be Age 1.

- Otolith 10:

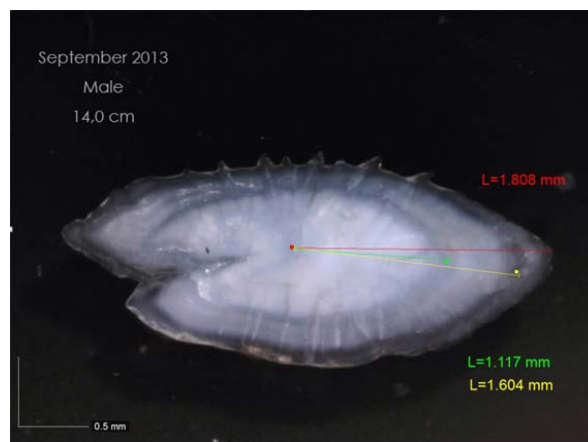


Date of capture: September 2014 **Length:** 136 mm

Age determination: age 2

Otolith Interpretation: Green measurement represents growth in first year of life while yellow measurement represents growth in first two years of life. The interpretation would be Age group 2, or Age group 1 if we consider yellow dot as a “spawning” check. In this case, the preferred interpretation is for Age 2 (because the second ring is strongly marked and well placed, matching the typical growth pattern in many areas).

- Otolith 11:

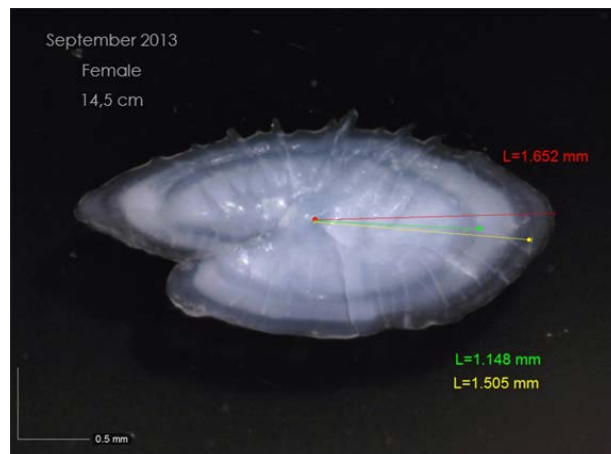


Date of capture: September 2013

Length: 140 mm

Age determination: age 2

Otolith Interpretation: Green measurement represents growth in first year of life while yellow measurement represents growth in first two years of life. The interpretation would be Age group 2, or Age group 1 if we consider yellow dot as a “spawning” check. In this case, the preferred interpretation is for Age 2 (because the second ring is strongly marked and well placed matching the typical growth pattern in many areas).

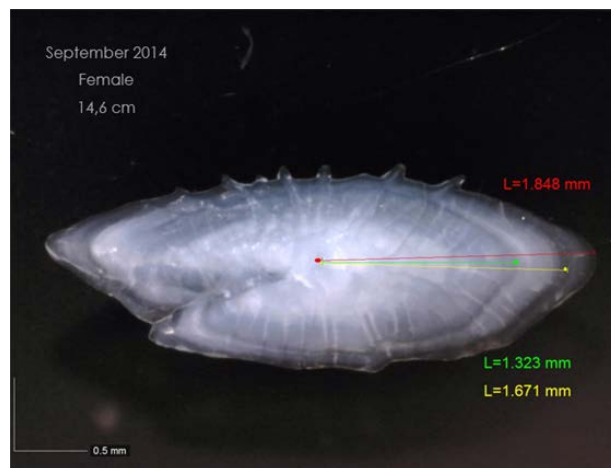
- Otolith 12:

Date of capture: September 2013

Length: 145 mm

Age determination: age 2

Otolith Interpretation: Green measurement represents growth in first year of life while yellow measurement represents growth in first two years of life. The interpretation would be Age group 2, or Age group 1 if I consider yellow dot as a “spawning” check. In this case the preferred interpretation is for Age 2 (because the second ring is strongly marked and well placed matching the typical growth pattern in many areas).

- Otolith 13:

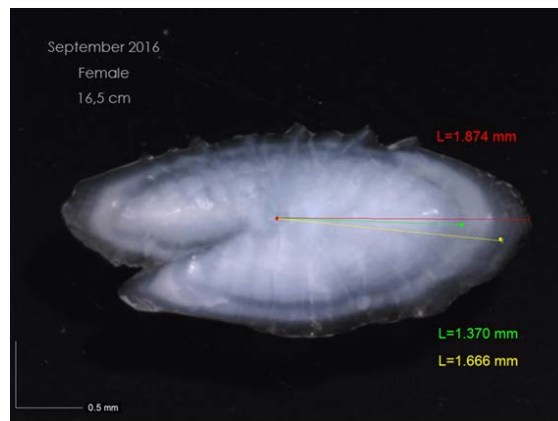
Date of capture: September 2014

Length: 146 mm

Age determination: age 2

Otolith Interpretation: Green measurement represents growth in first year of life while yellow measurement represents growth in first two years of life. The interpretation would be Age group 2 or Age group 1 if we consider yellow dot as a “spawning” check. In this case, the preferred interpretation is for Age 2 (because the second ring is strongly marked and well placed matching the typical growth pattern in many areas).

- Otolith 14:



Date of capture: September 2016

Length: 165 mm

Age determination: age 2

Otolith Interpretation: Green measurement represents growth in first year of life while yellow measurement represents growth in first two years of life. The interpretation would be Age group 2, or Age group 1 if we consider yellow dot as a “spawning” check. In this case, the preferred interpretation is for Age 2 (because the second ring is strongly marked and well placed matching the typical growth pattern in many areas).

7.2.9 Aegean Sea

Conventional Birthdate: 1 June

The otoliths presented in this exchange from the sampling area GSA 22 Aegean Sea, were collected during sampling campaigns on commercial purse-seine fishery in June and in September of 2014. Conventional birthdate in the area is considered as the 1 June, but a suggestion to move it at least at the 1 July, if not even at the 1 January, has been proposed after this workshop.

The otoliths of the GSA 22 used during the 2014 Exchange, presented 84% of cases where agreement was less than 80% and a 16% of cases where agreement was more than 80%. The difference age range was maximum for one age-class disagreement (94%) followed of two age class (6%).

The higher discrepancy in reading was among Age 0 and Age 1 and the main confusion was probably due to the misuse in the Rule of age assignment according to the birth date, as in the following examples.

In the pictures: Red cross shows one hyaline ring at the edge; Green arrow a hyaline discontinuity and the green range a potential autumn - winter – spring region of growth.

- Otolith 1:



Date of capture: 20/06/2014

Length: 90 mm

Age determination: 1

Otolith Interpretation: The otolith (1_49) presented in this picture, was collected in June 2014. It was considered Age 0 from 50% of the readers, but 9 readers, among them 5 Experts, did not agree with the modal age, indicating Age 1. Here the disagreement was due to the interpretation of the edge and that it has no strong winter hyaline mark before its edge. The interpretation was that there was a wide wintering zone without any clear hyaline mark between almost half the otolith and its edge defining it all the first winter ring, so for the conventional birthdate of 1 June was Age 1. In any case this would be a good candidate for micro-increment analysis to distinguish between age 0 and age 1 fish.

- Otolith 2:



Date of capture: 20/06/2014

Length: 105 mm

Age determination: Age 1

Otolith Interpretation: The otolith (1_18) shown in this picture, also collected in June 2014, presented an agreement of the 72% on Age 1, while 5 readers, among them 2 Expert readers, did not agree with the modal age. In this case Area readers both consider the otolith of Age 0; however modal age for Expert readers was considered as 1. Here the confusion was due to the misunderstanding of the growth pattern, the edge recognition and definitely the confusion on conventional birth date. The preferred interpretation for Age 1 corresponds to the same scheme described for the former otolith (1_49). This is the interpretation already provided for this otolith in section 8.1.

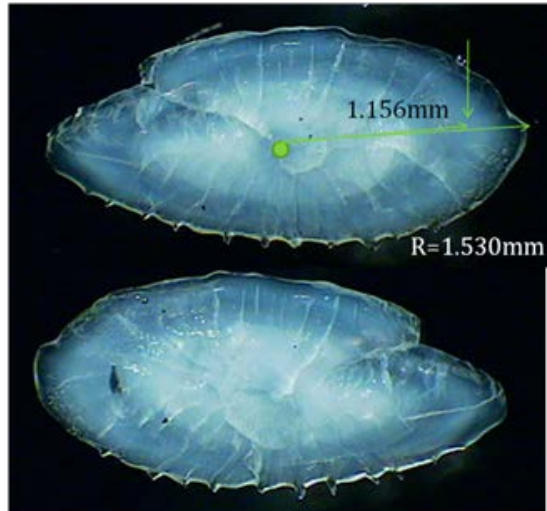
The two examples above are very similar and rather difficult to achieve a convincing interpretation because they have no clear hyaline marks within the otolith other than the edge (although some discontinuity between the inner white area and a secondary more grey area till the edge does exist). Even the edge in Otolith picture 2 is not markedly hyaline. Furthermore if the hyaline edge would correspond to the first winter ring then these otoliths would have not grown till June (so they would show abnormal growth pattern compared to other areas). Both otoliths are good candidate for micro-increment analysis to distinguish between age 0 and age 1 fish.

Reading exercise on otoliths of the GSA 22 presented a very high percentage of different age reading especially between Age 0 and Age 1, due to a poor marking of winter rings and consequently insufficient typical annual growth pattern recognition and insufficient criteria regarding the otolith edge that can be expected to be seen along the year in this area. In addition, in this area many otoliths were sampled during June and there was a strong misunderstanding if they should be considered Age 0 or Age 1, since 1 June was adopted as conventional birthdate in the area. The aspect of these otoliths was normally of Age 0 (rarely they marked a neat winter ring, but due to the birthdate on 1 June, they should be considered Age 1 (because a fish of about 9–10 cm in June ought to be born earlier than June and hence they cannot be age 0; as in the examples otolith pictures 1 and 2 above). Validation studies of the first annual increment deposition through the study of the daily growth based on otolith microstructure analysis should be undertaken in the area. This will be a very important step for GSA 22 since it could shed some light, especially to validate the real Age 1 class. Moreover, the conventional birth date set on 1 June cause an ulterior difficulties in considering the “first” and “second” semester, because with such birthday the first semester should start on 1 December of the previous year and end on 31 May of the following one, while the second semester should be considered from the birthdate on 1 June until 30 November. A suggestion to move the birth date of the species in the area at least on 1 July, if not even on 1 January, has been proposed after this workshop. Furthermore if the otoliths shown above (Otoliths pictures 1 and 2) were born during winter, then there would a strong advantage moving the birthdate to January as that will make easier the recognition of homogeneous age classes all throughout the year according to the number winter marks.

Finally, thanks to the reading exercise done during the Workshop on otoliths images from the different areas, among them GSA 22, it was possible to clarify the typical aspect of an otolith of Age 1 at the end of the year, as shown below.

Typical age 1 at the end of the year

- Otolith 3:



Date of capture: December of 2014

Length: 123 mm

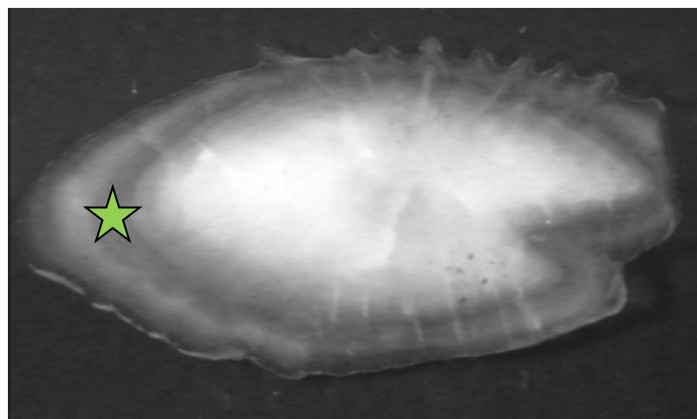
Age determination: Age 1

Otolith Interpretation: Green arrow shows one hyaline ring. Age interpretation is 1-year-old. This individual was caught in the second semester (in December) and there is only one clear inner hyaline ring. The edge is also hyaline and would correspond to the formation of the second winter hyaline ring, though this will not be taken into account for the assignation of the Age during the second half of the year.

7.2.10 Tunisian area

Conventional Birthdate: 15 July

- Otolith 1: GSA 12



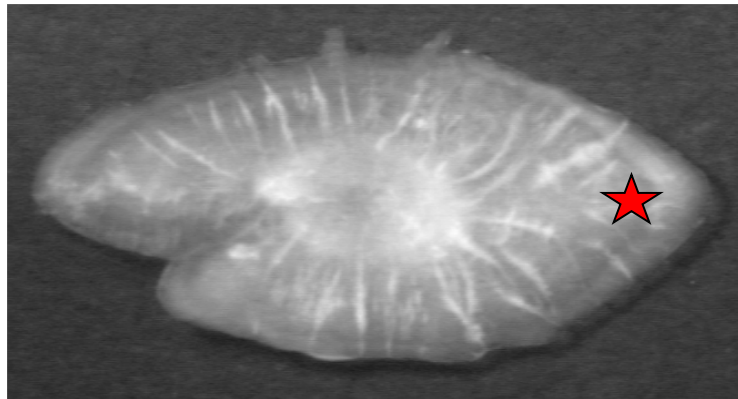
Date of capture: October

Length: 131 mm

Age determination: Age 1

Otolith Interpretation: One winter mark well marked. Workshop interpretation: Age 1 (A1 the green star + Check +hyaline edge).

- Otolith 2: GSA12



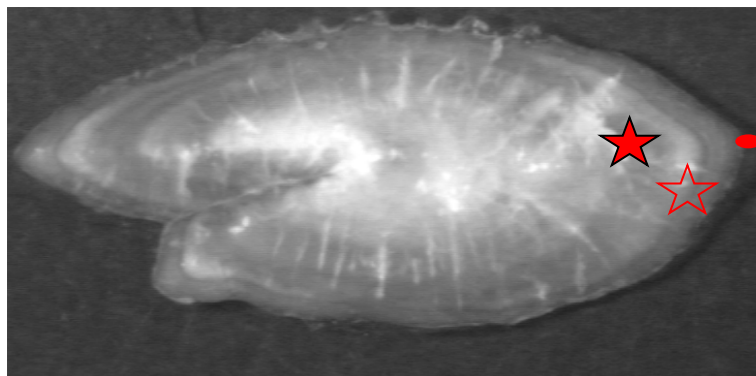
Date of capture: April

Length: 98 mm

Age determination: Age 0

Otolith Interpretation: To differentiate the winter mark from check we must make a microstructure analysis. Workshop interpretation: Age 0 (the red star as first winter ring + Opaque edge).

- Otolith 3: GSA12



Date of capture: May

Length: 115 mm

Age determination: Age 0-1?

Otolith Interpretation: Two different interpretations are possible for this otolith:

- a) Only one winter ring and opaque edge: Age 0 (a check C08+ empty red star as first winter ring+ growth of the year)
- b) Doubled winter ring and opaque band and hyaline ring at the edge. Age 1 (the red star as first winter ring+ spawning check + second winter hyaline ring at the edge).

It would be convenient to apply micro increment analysis to differentiate Age 1 from older (i.e. the first real winter ring from checks).

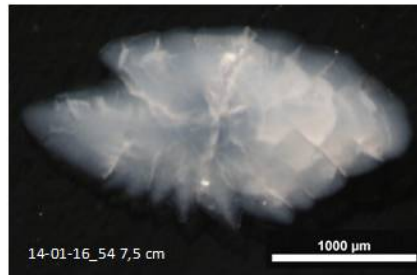
7.2.11 North of Morocco

Conventional Birthdate: 1 January

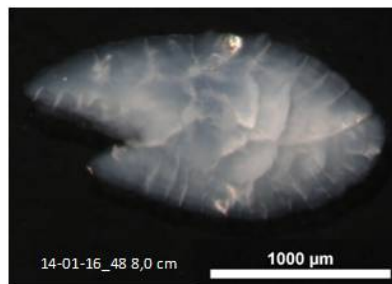
Spanish small pelagic purse-seine fleet fishing off North Morocco carry out 1 or 2 days fishing trips. Biological samplings of anchovy are routinely carried out on a monthly basis and 5 pairs of *Sagitta* otoliths of each length class are extracted to be later read in order to estimate the age of the individuals. It is assumed that anchovy's otolith grows forming pairs of rings. One opaque ring and one hyaline ring would complete an annual growth cycle. The formation of the hyaline annual mark occurs in winter, but otoliths of anchovies living in this area do not show well-marked hyaline rings, fact that makes difficult the age estimation.

First semester

- Otolith series 1:



Length: 75 mm



Length: 80 mm

Date of capture: January, 2016.

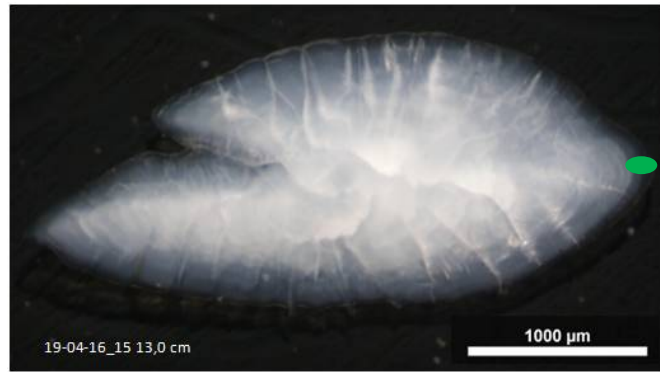
Age determination: 1 year old.

Otolith interpretation: These anchovies were born the previous summer. We assume 1 January as the birth date so they are age 1. Although no hyaline edge can be seen yet.

- Otolith series 2:



Length: 115 mm



Length: 130 mm

Date of capture: January and April, 2016.

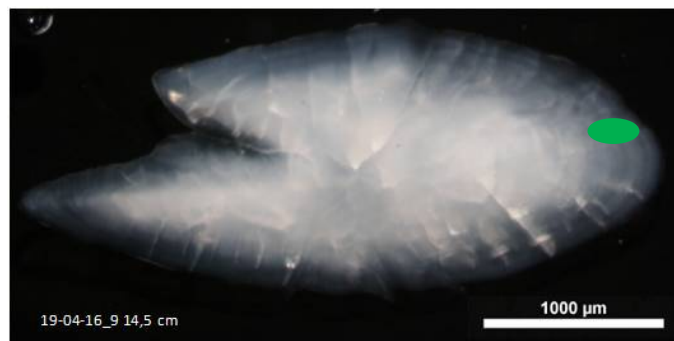
Age determination: 1 year old.

Otolith interpretation: It is possible to identify a weak translucent zone prior to the edge and a hyaline edge that we assume they both combined to correspond to winter ring.

- Otolith Serie 3:



Length: 140 mm



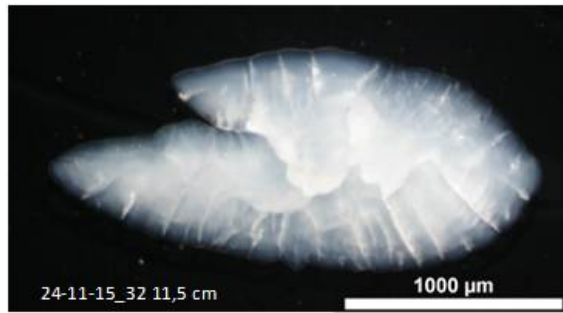
Length: 145 mm

Date of capture: April 2016.

Age determination: 1 years old.

Otolith interpretation: Two or more weak-marked hyaline rings that we assume to be a multiple marking zone. So we estimate that all these specimens are age one.

Second semester
- Otolith serie 4:



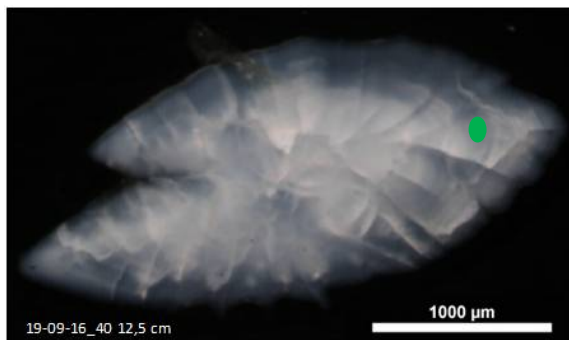
Length: 115 mm

Date of capture: November 2015.

Age determination: 0 years old.

Otolith interpretation: Although the size of the otolith is quite big, there are no marks. So we assume that next coming winter would have been the first lived winter and that the age is 0 year old.

- Otolith serie 5:



Length: 125 mm



Length: 140 mm

Date of capture: September 2016.

Age determination: 1 year old.

Otolith interpretation: A weak translucent zone is assumed to be a winter ring, although it is not well-marked. Moreover in the second otolith the spring-summer growth formed after the winter ring seems to be not so big as expected.

The first otolith the first marked ring could be a Check C06. It might be tested through microincrement analysis of the otolith, as it will discriminate between age 0 and age 1.

- Otolith Serie 6:



Length: 145 mm



Length: 155 mm

Date of capture: September 2016.

Age determination: 1 year old.

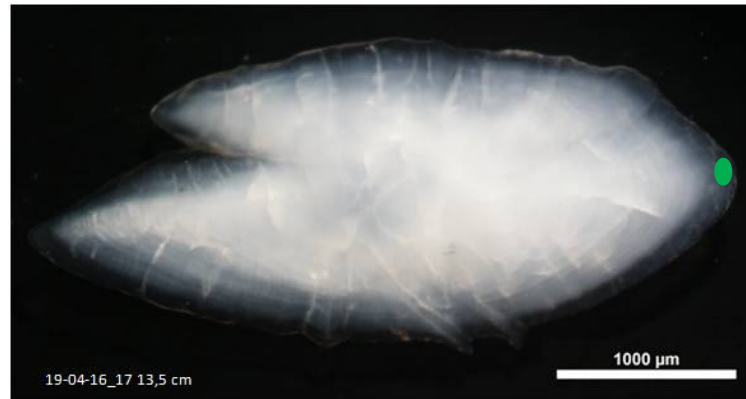
Otolith interpretation: Two or more weak-marked hyaline rings that we assume to be a multiple marking zone. So we estimate that all these specimens are age one. Although the spring-summer opaque growth formed after the first winter ring seems to be not so big as expected.

Here, as before, the potential for the first marked ring of being Check C08 might be tested through microincrement analysis of the otolith, as it will discriminate between age 0 and age 1.

The deposition pattern of a type of some otoliths, very opaque structure and no well-marked hyaline zones, can generate doubts to the reader if he suspects that hidden marks

may occur within the otoliths. However a straightforward interpretation and age allocation is obtained by admitting that there are no hidden marks.

- Here are some examples of typical otoliths with no marks:



Date of capture: April 2016.

Length: 135 mm

Age determination: Age 1

Otolith Interpretation: The winter hyaline ring in the edge.



Date of capture: April 2016.

Length: 160 mm.

Age determination: Age 1

Otolith Interpretation: The winter hyaline ring at the edge

8 Evaluation of quality of age data provided to the Anchovy Working Group Assessment

The result from Exchange reading exercise in 2014 reveals that there is a poor agreement and high CV in the age determinations (Table 6.1.1), not only at the overall global level (65% of agreement and CV of 58%), but also for the expert group (72% and CV of 52%). It is true that area readers tend to have a higher consistent age determination among them but in two of the three cases where such an increase exceed 80% of agreement (GSA 16 and 22) it was probably due to the fact that both local readers worked at the same institute. Therefore that did not guarantee higher quality in age reading *per se* in those two areas.

There is no clear international standard on the minimum level of agreement and precision of age determinations as inputs for assessments (Panfili *et al.*, 2002). In the update of the WGBIOP Guidelines for Workshops on Age Calibration (September 2015) it is recommended the formulation of species (and stock specific) target and threshold statistics as a tool for the evaluation of the quality of age readings: *“The statistics refer to the percentage agreement, the CV and the bias. The target value is the value you would like to achieve and know is possible based on exchange and workshop results. The threshold value is the minimum value required before a reader is qualified to supply data to working groups² and can if necessary be derived by discussion between expert readers. Usually, a CV of 5% is set as a threshold for sufficient data quality (Campana, 2001)”*. According to the 2014 exercise there was no area where such a precision was attained, even if restricted to the local area readers. The area of highest agreement and lowest precision both for the expert team and the local area readers was the Bay of Biscay but even in that case the CV of the area readers was 11% across all ages. Historical results for this anchovy shows that the overall level of precision is high, particularly for ages 0 and 1 (with agreements higher than 93% and CV lower than 10% and negligible bias), while for older anchovies precision levels are poorer (but agreements still higher than 80% and CV around 10–15%). In many studies about age quality it is found that typical CV lies between 0% and 16% (median about 7%; Campana, 2001). For anchovy and taking as reference the Bay of Biscay anchovy where several workshops and exchanges have regularly taken place (since 1989) and age validations are achieved, we think that thresholds could be set at about agreements around 80% and CVs around 20% across ages, and targets should be for agreements above 90% and CV of 10% or less. Given the difficulties of the anchovy otolith readings in many places of the Mediterranean Sea we consider that the above proposal for threshold levels could be a good starting point for a minimum required for otolith readers, while the target would be an achievable goal for European anchovy.

Given that there is no agreed collection of otoliths by areas, it is hard to assess the actual quality by areas but taking those of the experts as a rough reference, Table 6.1.1 shows that all agreements are below 80% (except Areas 7 and 8) and CVs above 20%. Therefore the quality will be probably moderate, too poor for most areas. Only areas 7 and 8 of ICES show agreements above 80% (though CVs above 20%). And we have to move to the local area experts of area 8 (from several institutes) to achieve levels above 90% of agreement and a CV of about 11%.

Over all readers the dispersion in age determination is high (Table 7.1.1) usually modal discrepancies cover a range of 2 or more ages for all areas. This for a species with age composition comprising only about 5 age classes (from 0 to 4) is a rather high dispersion.

² It means for an Assessment working group.

Bias seemed not relevant across ages or areas (Table 6.1.1), but the analysis of the 2014 exchange exercise showed that there seemed to be a difference of criteria among some readers of the Mediterranean and the Atlantic areas. In particular Mediterranean Readers 9, 10, 18, and 19 and Atlantic Reader 16 tend to age older the fish than the rest of the readers. During the Workshop it was corroborated that there was tendency for some readers in several areas to count almost every mark as a true annual winter zone and hence to assign older ages than the rest of readers. Through the discussions in the workshop it was also corroborated that most likely this was not a correct procedure as it resulted in rather incoherent age allocations in allowing very different growth patterns among examined anchovies of the same areas and even of the same sample.

A recent analysis in the assessment GSA working group revealed cohorts could not be well followed in the matrix of catches-at-age for several GSA regions, particularly in the Eastern Mediterranean areas (GFCM_FAO_2016), where the following remark appeared *“The WG highlight that there are differences in the ALK between these stock and those for similar stock in the Mediterranean Sea. Therefore the WG recommends an in depth analysis of the age reading”*. This supported the perception of inconsistency in age allocation with the tendency to admit every mark as true winter zone.

All this suggests the existence of bias in some age determinations for different areas. However, the absence of an agreed collection of otoliths by areas prevents a proper evaluation of such bias or the elaboration of matrixes of age allocation errors. In order to give an idea about the magnitude of changes in age composition which might originate from the different ageing procedures, readers should have a look at Table 6.1.3 where the different age composition (percentages) obtained by every reader can be checked, highlighting here what the local area readers obtained, during the 2014 exchange exercise. Certainly, the potential changes in age composition would be relevant.

The discussions on examples among the set of otoliths which generated the discrepancies in the age determination lead to conclude that there were two major sources of disagreements: a) Divergent otolith interpretation and b) wrong application of the age allocation Rules.

Regarding reason a) Divergent otolith interpretation; this refers to the evaluation of different interpretations of the marks, growth bands and edges for their conformity with the expected growth pattern of the anchovies, seasonal formation of the otolith by ages and most common checks, so that most plausible interpretation is retained. Section 7.1 and 7.2 above give several examples of discussion on the potential interpretation of otolith structures resulting in different age determinations. Further examination of these elements for the interpretation of otoliths and correct age determination is presented in the following two sections (10 and 11).

By the end of the workshop most of the readers agreed that there was a strong need of applying a global interpretation of otoliths, in growth pattern, edge recognition and identification of checks before counting marks for age allocation. Obviously changes in age composition would be substantial in several areas towards younger ages if, as presumed, correct interpretation of otoliths would lead to reduce the number of admitted true winter translucent zones. Noticeable changes may occur in regions around Tyrrhenian, Ionian, Sicily, Aegean Sea and in 9a (Central area), for which area readers produced oldest age compositions than the other readers in the 2014 exchange, but also this may happen in the Adriatic and Tunisian waters, as realized during this workshop.

Regarding reason b) wrong application of the age allocation Rules; It was evident during the workshop that for the birthdate first July (or first June) the age determination rule was not being correctly applied during the first half of the year (from January to June).

This was partly due to the way it was described (written) in some texts of the 2009 WKARA report. As a result of this some readers gave ages during the first half of the year which were one year older than what should be done for particular otoliths showing hyaline edges. Correction of the actual writing of the rule is made in section 11 below. Certainly, correct application of the rules for age allocation would also lead to a shift of the anchovy catches-at-age towards younger ages in the first half of the year.

Another reason which might be behind the lack of coherent tracking of cohorts in the matrix of catches-at-age in some Mediterranean areas is the way the half year catches-at-age are collapsed into annual catches-at-age for assessment purposes. As a rule of thumb, assessments based on year time-steps need joining consecutive half year periods of catches-at-age with every annual cohorts (year class) being allocated to the same age classes. For the ageing methods assuming birthdates at mid-year (either 1 July or of June), every year class becomes one year older in the middle of the year at its birthdate. Thus for an assessment running in annual time-steps going from January to December first and second half of the year cannot directly be merged into homogeneous age groups unless age classes of the first half of the year are pooled with the one year older age classes of second half of the year. Assessment modellers of the assessment working group should make sure that the age classes merging procedure has been correctly applied in the past, so that the same year classes were pooled together. If this procedure was not taken duly into account, so that the same age classes of the first and second half of the year were directly added up, then successive year classes would be confused and wrongly merged into the same age class in the matrix of annual catches-at-age. If this has happened this would also (in addition to the former reasons given above) imply a loss of the year class (or cohort) signals in the series of catches-at-age. Hence the Workshop recommends verification that the proper procedures were followed in the past to generate the annual catches-at-age inputs for the assessment working groups.

In summary, following the workshop discussions there has been a progressive change in the perception of the growth pattern applicable to these anchovy otoliths in many areas (becoming closer to that applied in the Bay of Biscay), and a general awareness of the frequent occurrence of checks, which led to some revisions of the otolith interpretation and assigned ages. Furthermore, there have been evidences that the age determination rule for the birthdate at the middle of the year has in some instances been inconsistently applied for the first half of the year. These evidences lead to concluded on the need to review past age determinations.

As a corollary of the former statements, Intercalibration ageing exercises by areas (for the different countries taking part in the age reading of anchovies from the same stocks) are still strongly required.

9 Analyse growth increment patterns in anchovy otoliths (ToR b)

The starting position at the beginning of the workshop was that growth patterns of otolith vary quite much between different areas. This was presumed according to the relevant discrepancies in age determination between readers for the different areas under study and according to the general opinion resulting from the former WKARA report (WKARA 2009) where it was mentioned that: “A large proportion of variability, observed in the anchovy growth between areas, is explained by the changes in the habitat conditions, namely chlorophyll concentration and temperature. The estimated parameter K of the Sicilian anchovy was at the lower end of the range observed for this fish species in different areas. Average chlorophyll concentrations, and sea surface temperatures derived from satellite images for each area, explained the 88% of the variance observed in the growth of the anchovy between the areas (Basilone *et al.*, 2004). The common opinion among readers and participants to the WKARA was that based on the aforementioned variability of the growth pattern, also within the same area, a general role or guideline is not advisable.”

However as we progressed on the discussions and presentations during the workshop it became evident that the differences may not be so relevant as initially believed, and that a general growth pattern could emerge rather common to all areas, as explained below in this section.

On the other hand major differences may appear however on the intensity of the winter hyaline zones, so that the more to the North an area is placed, the greater the intensity of winter translucent zones (Bay of Biscay, Gulf of Lion, Adriatic). The most southern regions seem to lay down less pronounced winter hyaline zones, showing less contrast between hyaline and opaque zones, and therefore increasing the difficulties for identifying true winter rings from other hyaline marks or checks appearing quite often in these otoliths (Morocco, Tunisia, Sicily). Finally Gulf of Cadiz, Ionian, Southern Thyrrenian and Aegean seas seem to show an intermediate intensity of hyaline marking.

In all areas readers identify checks and therefore in order to improve the age determination readers should become familiar with the most common checks in their area. Several of the exercises which are suggested, as follow up actions of research, from this workshop address the issue of differentiating checks from true winter hyaline zones.

General Pattern of annual growth

Prior to any discussion, summary descriptions of the major pattern of annual growth (a), edge formation along the year (b) and typical checks (c) in the otoliths of the European anchovy in the different areas were presented, along with the procedures for age determination.

The procedure for anchovy age determination European anchovy is the one adopted in WKARA 2009 and prior workshops according to the available validations by Uriarte *et al.* (2007; 2016), Aldanondo *et al.*, 2016; Giraldez and Torres (WD 2009) and Donato and La Mesa (WD 2009), Millan and Tornero (WD 2009) (the WD are consultable in the share point website: <http://groupnet.ices.dk/WKARA/default.aspx>).

The method of age determination is based first on the interpretation of otoliths according to the prior biological knowledge of the annual growth pattern of the anchovy otoliths, of the seasonal growth of otolith edge by ages and of the most typical checks. The selected interpretation would be the one best accommodating to the prior biological knowledge. According to the interpretation achieved, the number of past winter (translucent) zones

will be used to apply the rules for allocating ages according to the assumed birthdate for the population being studied.

Here below follow the summary of our base knowledge of the growth pattern:

a) Typical annual growth of the otoliths is established, by which annulus width during the first, second and third years of life (corresponding to 0, 1, and 2 years old groups) decreases progressively. Oldest ages (3-and 4) present more similar widths (but still slightly decreasing) compared to the one experienced at previous ages. Certainly this pattern respects the general principle by which year growth increments in otoliths gradually decrease (Morales-Nin and Panfili, 2002), but the particular way anchovy respect this pattern is the first general scheme age readers should be aware of for the examination of these otoliths.

In the Bay of Biscay typical annual growth of the one and two years old anchovies diminishes to about a half and a third of that occurring in their previous ages respectively, though individual variability is high (CVs of 44% and 33% respectively). In subsequent ages, opaque growth still diminishes but to a lesser extent than the trend shown during the first three years of growth. As such, at age-3 opaque growth is still about half of that produced at age-2 and subsequently relative reductions are even less intense. This pattern seems to be applicable to many areas in the Mediterranean Sea and the examples shown along the report are not in contradiction with the general pattern. However in some of these areas there are some otoliths for which the decrease in growth increment at age 1 compared to that achieved at age 0 might be more pronounced than in the Bay of Biscay (decreasing to already to about a third) as for example shown by the pictures from the Tyrrhenian sea area (in section 8.2.5) or as in the Strait of Sicily (Otolith 6 in section 8.2.6). In those cases the growth width at Age 2 looks very similar to that at Age 1 (barely noting any decrease).

b) Maximum otolith growth (opaque zones formation) takes place in summer, and it decreases in winter (hyaline zones formation). However, in some areas, the starting of the opaque edge during spring changes with ages, being remarkably sooner for those at their first year of life (just after winter) than at older ages. For instance in the bay of Biscay (with conventional birthdate at 1 Jan) it is shown that in spring the one year old anchovy (just overpassing their first winter) have typically already started the deposition of the opaque growth zone, whereas 2 years old or older fish have mostly hyaline edges (or at the end of the spring in early formation of the opaque ring).

The general seasonal edge formation of otoliths being mostly hyalines in winter has been also confirmed in the Northern Alboran Sea and in Tunisian waters (see sections 5.3 and 5.9 respectively). However in area 9a, both in the 9a South and in the 9a North, the analysis shown in section 5.2 suggest to have a contrary seasonal pattern, with formation of hyaline edge mostly in summertime. This may require further analysis, since it seems to indicate that the growth is greater in winter than in the summer. Potential explanations worth considering are the potential of summer upwelling regimes (of cool water) affecting otolith growth, spawning or false checks and/or difficulties in proper hyalinity characterization of the edges.

On the other hand the early starting of the opaque growth for the 1 y.o. fish (since march) seems to be confirmed for almost every area as exemplified by the many pictures showing the typical opaque growth of the edge of the 1 y.o. during Spring (from Cadiz, 9.a to Tunisian waters, etc.)

c) Typical checks occur mainly before and after the first winter ring is formed, during ages 0 and 1 of anchovy. The check before the true hyaline winter ring is generally of a

faint and poorly defined structure. Preliminary results based on microstructure analysis suggest that this could actually be a check (laid down at about 0.8 mm from the nucleus corresponding to about 90 days old fish caught in autumn) (Hernandez *et al.*, WD 2009). But the possibility of this check corresponding to the first winter ring cannot be discarded for fish born late in the spawning season (as in September or October- something possible due to the protracted spawning season seen in many of these areas). The most typical check formed after the first true hyaline zone is formed during late spring or summer in many of the 1 years old anchovy at the peak of their first spawning period, which is considered to be a spawning check. According to its position in relation to the total expected annual growth checks are named C15 or C18 if laid down around 50% or 80% of expected annual growth.

The number of check rings (not winter translucent zones) seems to be highly variable both between individuals of a given cohort and between cohorts (Uriarte *et al.*, 2016). Usually checks tend to be weaker or more diffuse than true annual rings and often they are not completely formed all otolith around, their position will often differ from the expected position of the true annual rings. Though controversial marks in otoliths are in some cases encountered and some examples are provided in this report.

10 Update Age reading protocol for European Anchovy (ToR b)

Recommendations for anchovy otolith preparation and examination:

- 1) After extraction otoliths are washed thoroughly, dried, mounted and preserved in a synthetic resin ("Eukitt" or "Entellan") or stored dried. For posterior micro increments analysis dried storing is compulsory.
- 2) The observations of entire otoliths are made under reflected light against a black background using dissection microscopes with 20X or -25X magnification.
- 3) Magnification might be increased near the otolith edge to improve the discrimination of narrow hyaline or opaque edges in old individuals.
- 4) For each otolith recording of the following information is recommended, and in cases of exchanges and workshops collection of that information would be mandatory: the number of true hyaline winter rings, edge type, age assigned and readability (Age quality AQ scoring 1 – easy to age, 2 – difficult to age, 3 – practicably unreadable or very difficult—WKNARC2011), as well as if false rings (checks) were detected and whereas for instance C0+ or C1+ or C2+).
- 5) The nomenclature that has been adopted in this report could be of general utility for all anchovy readers:
 - a. For winter rings they are called according to the ordinal number of the winter period: as such first winter ring is called A1 and second winter ring is A2, etc.
 - b. In term of false rings or checks (not winter rings), Checks are typified and named (Uriarte *et al.*, 2016) with a C plus two digits according to the age of the fish when formed (first digit) and to the approximate relative position over the expected annual growth of the otolith at that age (second digit). For instance, the most typical checks formed during the first year of life (age-0) are named C05 or C08 because they are formed approximately at 50 or 80% of the expected annual growth of the otolith at age-0. Other typical checks are C12, C15 or C18, which correspond to checks formed at age-1 at ~20, 50 or 80% of the expected annual growth of the otolith for that age. Checks C12/15 refer to checks C12 and C15 together due to the difficulty of discerning one from the other when annual opaque growth is not complete and because they define a continuous range of relative positions.

In order to standardize the anchovy age assignments and to improve the age estimates, this Workshop adopted the following protocol:

Age determination is a two-step process consisting in:

- a) First: Otolith interpretation aiming at the recognition and counting the existing annulus in the otoliths (i.e. complete annual opaque growth bands and winter translucent zones); examination of the edge type and identification of checks, so that such interpretation is evaluated for its conformity with the biological base knowledge acquired on the annual and seasonal growth patterns of otoliths (and most common checks) for the species and population. At this stage not to look at the size of the fish: see the structure of the otolith and growth pattern.
- b) Second: Applying the rule for age allocation corresponding the conventional birth date adopted for the population under study (Figure 10.1):

- For a birthdate convention at first of January: The Rule is that age equals the number annulus (i.e. amount of complete annual opaque growth bands and winter translucent zones); this is equivalent to say that age equals the amount of past complete annual opaque growth bands; or that age equals the amount of winter hyaline zones observed in the otolith. Notice that for the first months of the first half of the year the last winter hyaline ring may be just being formed at the edge of the otolith, and if the rule is being applied just in terms of numbers of winter rings then this last ring should be counted for the age allocation rule. The Rule is valid for fish caught in any of the two halves of the year. Generally the Rule has been formulated as that Age equals the number of winter hyaline zones observed in the otolith, but notice that the formulation based on the amount of winter rings requires that the last winter ring is visible since the beginning of the year. But as this not always true it may be safer for otoliths at the beginning of the year to apply the age allocation rule based on the amount of past annual opaque growth bands.
- For a birthdate convention at first of July: the Rule for age allocation changes depending on the half of the year when the fish was caught: For the first half of the year, the age should equal the number of annulus (or winters hyaline zones) observed minus one. For the second half of the year age equals the number annulus (or past winters hyaline zones) observed in the otolith. All former comments for the other convention birthday equally apply here.

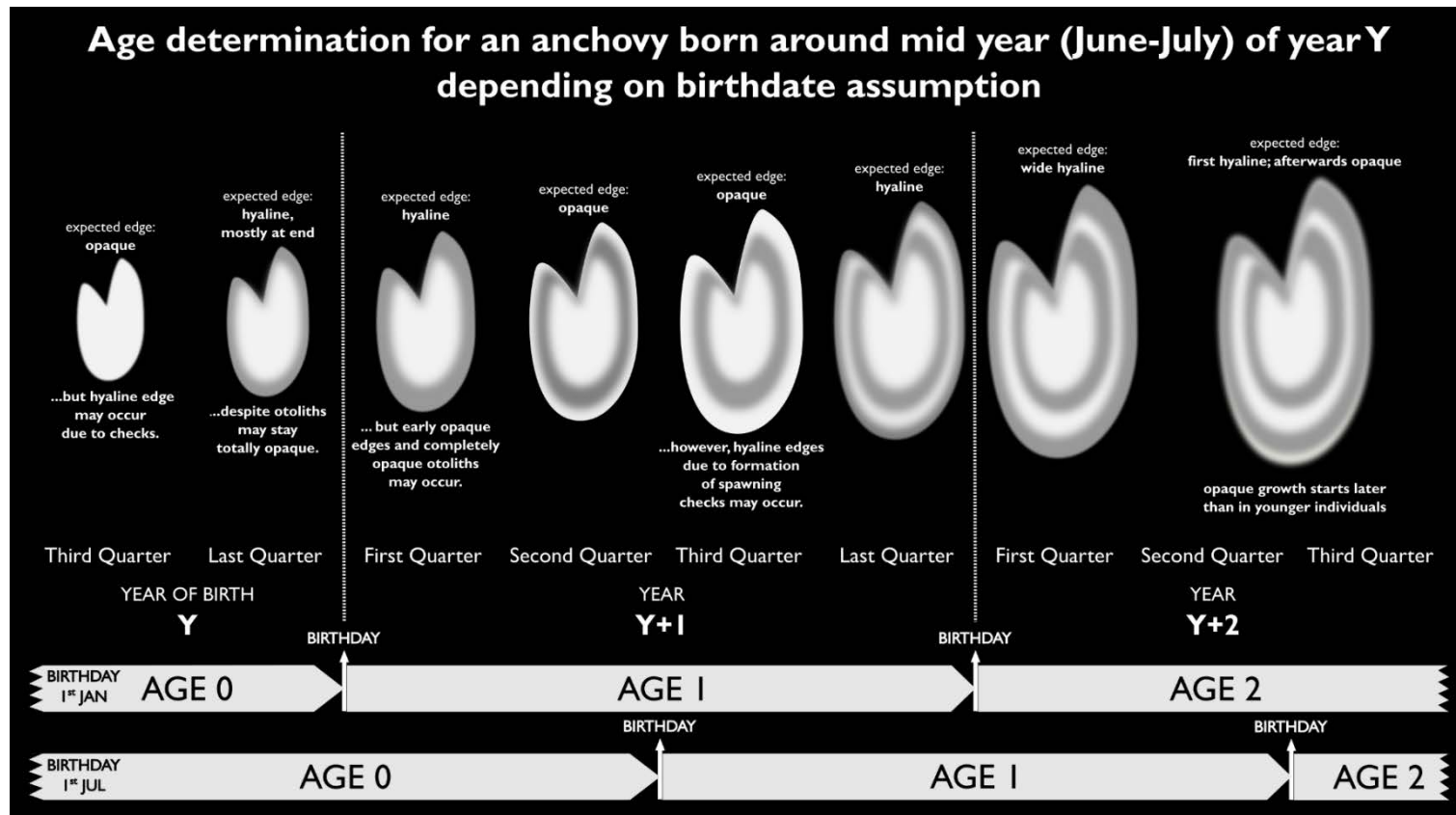


Figure 10.1 Synoptic representation of the anchovy otolith development in time and the different age allocation according to the two conventional birth dates on 1 January and on 1 July.

Comments for interpretation

Interpretation of Hyaline edge:

- 1) During some months of the first half of the year the last winter hyaline zone may be present (or being formed) just at the edge of the otoliths, such winter hyaline zone at the edge is to be included in the counts of past winter zones. Rarely such a hyaline zone at the edge may be insufficiently formed at the beginning of the year (Jan-March), but as the former complete year opaque growth band will be well visible the winter hyaline zone could be presumed and counted for application of the rule for age allocation (provided no doubt exists that the previous opaque band corresponds to a complete annual opaque growth band).
- 2) During the second half of the year opaque edges will be usually observed corresponding the opaque growth season of the year. Therefore, hyaline winter zones should be visible within the inner part of the otoliths. If the edge is hyaline then it is to be presumed that such edge will correspond to either a check (spawning or summer check) or to the early occurrence of an early growth detention during autumn, but such hyaline edge is not to be considered a new winter zone and in no way it should be counted among the past winter hyalines zones upon which age allocation rule applies.

Interpretation of weak (poorly marked) or multiples hyaline zones:

- 1) If a faint ring occurs at a distance where a true winter ring could be expected (either visually or compared with the typical mean radius of the 1st annual ring in other otoliths), the possibility that such a mark would correspond to a true winter ring could be evaluated for its conformity with the expected annual growth and edge type for the resulting age.
- 2) Some first winter hyaline zones may be composed of several close hyaline marks forming a cluster (two or more very close hyaline marks). Readers should be aware of such possibility for proper joint assignation to a single winter zone.
- 3) As mentioned above in section 9 readers have to be familiar with the most typical checks which usually occur before and after the first winter ring is formed, during age 0 and age 1 of anchovy (but see comments and examples above in this report).

On the use of radius measurements to discriminate between checks and the first true winter ring:

According to the work of Hernandez *et al.* (2013) to corroborate the position of the first hyaline check formed before their first winter annual ring, through the counts of micro-increments, it was suggested that hyaline marks placed at a distance from the core of less than 850 μm ($\pm 100 \mu\text{m}$) should be considered as a check (not as true winter hyaline rings). This was because those marks had been formed generally before a 120 days of life of the fish (and usually before 90 days). However in this workshop this R1 measurement Rule will not be undertaken by default to identify checks but just as auxiliary information in cases where the first hyaline ring is weakly formed and the readers have serious doubts about its reliability as true winter ring. This is suggested because for the protacted spawning seasons of these anchovy populations (usually reaching September or even October) there might be some few cohorts born in Autumn overpassing its first winter just at the age of 90 to 120 days. So visual interpretation of otoliths is favored over a quantitative examination of the marks.

11 About the conventions on birthdate

The WK noticed that the allocation of ages with the Rule for the birthdates at the middle of the year was in some cases not being well applied, leading to some miss allocation of age classes, particularly for fish caught in the first half of the year. So first the group made sure that everybody understood the way the rule should be applied for a correct allocation of ages and, in addition, the written rule has been reviewed in the current report (see above).

Assumed birthdate for fish is a convention which can be addressed from two points of view: a) from a biological point of view, in relation to the spawning time during the year or b) from an assessment point of view in relation to the time-steps in the assessment. From a biological point of view it can be of interest setting the birthdate around the peak spawning time (or just a bit earlier) so that fish will move into a new age class on average a year after they were born (about 365 days after). This implies defining year classes between subsequent spawning seasons (or between the beginnings of subsequent spawning seasons). From an assessment point of view, as assessments evaluate the decay of year classes in annual time-steps usually from January to December, it can be preferred that birthdate convention groups year classes in the same age classes all throughout the year. For this reason if assessments are carried on annual steps from January to December it can be preferred that birthdate is set on 1 January so that year classes (defined as the fish born from January to December) are allocated to the same age classes at any time of the year. Alternatively, if assessments are carried on annual steps from July (Y) to June (Y+1) it can be preferred that birthdate is set on 1 July so that year classes (defined as the fish born from July to June) are allocated to the same age classes at any time of the assessment year. Therefore the simplest approach is to set the birthdate at the bounds defining the year step used in the assessment, so that year classes are allocated to a single age classes for the year step for which the assessment is run.

In the Mediterranean areas birthdate conventions have been adopted following mainly the biological reasoning, and for most of stocks the birthdate convention is set on 1 July or 1 June. However assessments are currently run on annual steps going from January to December. This may cause some problems in the generation of annual catches-at-age coherently grouping year classes (defined for assessment purposes as the fish born from January to December).

For assessment models based on annual time-steps (from January to December) it might be better to assume that birthdate is 1st January. The reasons in favor of setting it on 1 Jan are: a) the application of the Ageing rule is simplified because a single unique criteria is applied all year around (for the two halves of the year, as explained in the previous section) and b) a single correspondence between year class and age class is achieved for the entire year period on which the assessment is run (January to December) and as a result of this annual catches-at-age are simply derived as the sum by ages of the catches-at-age of the first and second halves of the year (Table 11.1) (because a birthdate convention on 1 Jan makes that year classes are contained in the same age classes in both halves of the year).

Table 11.1: Proper elaboration of annual catches-at-age (from January to December) from Catches age estimates estimated on half year basis for a birthdate convention of First January, taking as example the estimation of catches-at-age in 2015.

Birth date in January 1st

Catches at age in 2015		Catches at age in 2015		
1st half	2nd half	Annual catches by year class	Name of Annual age class	Year Classes
NA	Age 0	Age 0 (2ndhalf)	Age 0	2015
Age 1	Age 1	Age 1 (half1)+Age1(half2)	Age 1	2014
Age 2	Age 2	Age 2(half1)+Age2(half2)	Age 2	2013
Age 3	Age 3	Age 3 (half1)+Age3(half2)	Age 3	2012
Age 4	Age 4	Age 4 (half1)+Age4(half2)	Age 4	2011
Age 5	Age 5	Age 5 (half1)+Age5(half2)	Age 5	2010

For assessment models based on annual time-steps (from January to December) assuming that birthdate is 1 July (as in the Mediterranean Sea) causes some difficulties which should be overcome: a) the application of the Ageing rule is not unique for the two halves of the year (but changes as explained in the previous section) and b) No unique correspondence between year class and age class is achieved for the entire year period on which the assessment is run (January to December) and as a result of this c) ALKs have to be constructed independently for the first and second half of the year and catches-at-age should be produced on half year basis using respective ALK (as age classes do not correspond to the same year classes for the two halves of the year) d) for the assessment running from January to December annual catches-at-age cannot be simply derived as the sum by ages of the catches-at-age of the two halves of the year; the addition of the catches-at-age has to take into account instead the shift in age classes occurring at the middle of the year for the same year classes (Table 11.2) (because a birthdate convention on 1 July makes that year classes are contained in subsequent age classes in the first and second half of the year respectively). Therefore, annual catches age 1 group, for instance, should merge Age 0 of the first half of the year with age 1 of the second half of the year. Annual catches age 2 group, should merge Age 1 of the first half of the year with age 2 of the second half of the year, and similar procedures apply to the remaining subsequent year classes (see Table 11.2 for a scheme of such procedure). Certainly for the Annual Age 0 group, only Age 0 of the second half of the year will contribute to that Age group.

Table 11.2: Proper elaboration of annual catches-at-age from Catches age estimates on half year basis for a birthdate convention of First July (for ALKs elaborated independently on half year basis), taking as example the estimation of catches-at-age in 2015.

Birth date in July 1st

Catches at age in 2015		Catches at age in 2015		
1st half	2nd half	Annual catches by year class	Name of Annual age class	Year Classes
Age 0	Age 0	Age 0 (2ndhalf)	Age 0	2015
Age 1	Age 1	Age 0 (half1)+Age1(half2)	Age 1	2014
Age 2	Age 2	Age 1(half1)+Age2(half2)	Age 2	2013
Age 3	Age 3	Age 2 (half1)+Age3(half2)	Age 3	2012
Age 4	Age 4	Age 3 (half1)+Age4(half2)	Age 4	2011
Age 5	Age 5	Age 4 (half1)+Age5(half2)	Age 5	2010

Another difficulty associated to the mismatch between the calendar associated to a birthdate convention at the middle of the Year and that of the assessment running from January to December can arise if catches-at-age are desired to be produced based on a

single annual Age Length Key: Such annual ALK cannot be constructed directly by merging by length classes the fish allocated to the same age classes in the first and second half of the year because of the shift in year classes at the middle of the year as explained above³. It is strongly recommended to avoid such practice and to generate instead independent ALK on half year basis.

In any case, for a small pelagic fish which shows an intense and continuous growth throughout the year, it is advisable to construct ALKs with a minimum temporal resolution of half year basis. Actually in the Atlantic area ALKs are produced on quarterly or half year basis. And this would be advisable for the Mediterranean areas as well.

The WK is aware that the present meeting is not the place to change the conventional birthdates; therefore the following message will be passed to WK on stock assessments, such as the GFCM and STECF WGs:

Warning for assessment: Assessment based on year time-steps need annual catches-at-age having a coherent and unique correspondence between age classes and year classes (for instance joining the consecutive half year catches-at-age with annual cohorts (year classes) being grouped into the same age classes). People in charge of preparing the annual catches-at-age for assessments should make sure that this procedure has been correctly applied in the past.

In addition it should be noticed that the age composition reported by surveys should be provided for stock assessment purposes on similar age classes (with age classes referring to the same year classes) as used for reporting the annual catches-at-age. For instance for a birthdate first July a survey on the first half of the year should report for assessment abundances at age corresponding to the Annual age classes applied to the annual catches age (as indicated in the Table 11.2).

³ To construct a correct annual ALK: Ages 0 of the first half of the year should be added by length classes with the ages 1 of the second half of the year and they all should be assigned to age 1 in the ALK. Analogously, Ages 1 of the first half of the year should be added by length classes with the ages 2 of the second half of the year and they should be ascribed to Age 2 class by length in the ALK, and so on for subsequent age groups... etc. And the Age 0 in such ALK will only be sustained by the Ages 0 of the second half of the year.

12 Update Otolith reference collection and a database of otoliths images (ToR d)

The reference collection of anchovy otoliths has been elaborated by a selection of otoliths images with more than 80% agreement of Expert readers from the 2014 otolith exchange (Table 12.1). Also few otoliths discussed and agreed during the WKARA2 were added in those areas where the collection of otoliths was small. The exact position of the winter hyaline rings (in the image marked with a red point) was determined by the reader with more experience within each relevant area. Ideally an agreed age collection should cover all age and length groups across the represented stocks. This agreed age collection was compiled by area and only based on the samples provided for the exchange thus limiting the temporal and spatial coverage.

The images are placed and available to interested experts on the Age Reader's Forum (ARF) which can be found at <https://community.ices.dk/ExternalSites/arf/default.aspx>. (while accessing the first time you will be promoted for permission and registration to ICES)

In future this agreed age collection should be expanded to include examples from all quarters and age groups for each stock.

Table 12.1. Anchovy otoliths with greater agreement of 80% of the expert readers from the exchange of 2014, by area

Expert readers		% Agreement >80		% Agreement 100%	
Set	N Total	N	%	N	%
Total	596	133	22	56	9
ANE_7	20	10	50	6	30
ANE_8	70	35	50	20	29
ANE_9.a	92	32	35	12	13
ANE_GSA01	70	11	16	2	3
ANE_GSA06	60	3	5	0	0
ANE_GSA07	38	14	37	7	18
ANE_GSA10	55	2	4	0	0
ANE_GSA16	66	6	9	0	0
ANE_GSA19	55	2	4	0	0
ANE_GSA22	70	18	26	9	13

13 Recommendations for future Research

Research by micro-increments counting on several selected otoliths by areas:

- Validation studies of the first annual increment deposition through the study of the daily growth based on otolith microstructure analysis should be undertaken in some areas. This will be a very important step in general for all areas, since it could shed some light, especially to distinguish between age 0 and age 1 fish when divergent interpretations appear.

Other Validations and corroboration methods:

- Validation studies could be made, especially studies of progression of length frequency modes throughout time, for tracking cohorts, as it is one of the most basic analysis which can provide reliable information on growth, particularly of young, fast-growing fish. Further, this method is low cost and takes advantage of data routinely obtained in fishery studies (length).
- Corroboration of inner consistency of age determination by following cohorts in catches and surveys.
- In addition Studies on the seasonal formation of hyaline and opaque edges are valuable to understand the dynamic of otolith formation by ages, although this is one of the least rigorous methods.

New Exchange in 2018

- As a calibration exercise was not performed during the WKARA2, it is recommended the realization of a small exchange to be carried out during 2018 in order to see if the update age reading protocol have been adopted by all readers and to see if the accuracy and precision continue to improve. A possibly dates for the exchange would be at the second quarter of 2018 (April-June). It is desirable that at least the same people attending the workshop take part of the exercise.

Recommendations to other ICES WG and GFCM:

- A) As there has been a progressive change in the perception of the growth pattern applicable to the anchovy otoliths in many areas which led to increase the agreement between readers and to revise some otolith interpretation and assigned ages. B) as there have been evidences that the age determination rule for the birthdate at the middle of the year has in some instances been inconsistently applied (particularly for the first half of the year). C) These evidences led to conclude on the need to review past age determinations. Although this task should be delayed until running the 2018 exchange, so as to be sure that all readers apply the protocol and the current criteria of this workshop coherently, since current criteria would change the otoliths interpretation and the age determination in many areas. The potential changes in age composition can be relevant.
- Inter-calibration exercises by areas (for the different countries taking part in otolith age reading on the same stocks) are required. This becomes compulsory for regions where several countries exploit the same stock. This is in agreement with point 38 of the Report of the Working Group on Stock Assessment of Small Pelagic species (WGSASP) Rome, Italy, 23 November – 28 November 2015 (GFCM-FAO, 2016).

- The WK put in doubt the convenience of having midyear birthdates, because the mismatch it generates between the calendars associated to a birthdate convention at the middle of the Year and that of the assessment running from January to December. As a result of this there is a no unique correspondence between age groups and year classes for the year time-step on which the assessment is run. The WK considered that it might be preferable adopting the birthdate of First January for all areas because a) the application of the Ageing rule is simplified, as a unique criteria is applied all year around (for the two halves of the year), and b) a single correspondence between year class and age class is achieved for the entire year period on which the assessment is run in most areas (January to December) and as a result of this annual catches-at-age would be derived directly as the sum by ages of the catches-at-age of the first and second halves of the year.
 - o Details for the correct application of Ageing rules and elaboration of annual catches-at-age as a function of the birthdate convention are provided in the report.
 - o For the areas where birthdates are placed at the middle of the year, and hence the same year classes belong to different age classes in the two halves of the year, the Workshop recommends to Assessment working groups to verify that proper procedures were followed in the past to generate the annual catches-at-age inputs for the assessments, so that successive year classes were not unduly merged.
- It is recommended that, as far as possible, only the age readings of the most expert readers are used for the assessment inputs and second that new readers pass a training processes from validated set of otoliths of the area they have to work with. WKARA2 suggest threshold values of agreements around 80% and of CVs around 20% in the training process as a minimum for age readers to be operative to deliver inputs for assessment.
- Production of a collection of age validated otoliths by areas (or at least of agreed age determination by experts) is recommended for the purposes of helping in the training of new age readers.
- And finally it is also recommended to have regular exchanges, both internally and externally, to learn and to improve the agreements between readers across and within areas.

14 Conclusions

- Indirect validations (marginal increment analysis, length frequency analysis, progression of year classes, Daily increments between annuli) for the annual age determination of European anchovy have been only applied in some areas/stocks, but the majority of works attempting to validate annuli of anchovy apply the qualitative method of marginal increment analysis, one of the least rigorous methods. So far, there are only two areas/stocks (Bay of Biscay and Northern Western Mediterranean) where more accurate validation methods have been used and published (Morales-Nin and Pertierra, 1990; Aldanondo *et al.*, 2016; Uriarte *et al.*, 2016). There are several areas/stocks for which validations for anchovy annual age determination are lacking. Validation of the age determination procedures should be mandatory for these areas/stocks to assure the quality of the age structured inputs for the assessments.
- In the 2014 Exchange the overall agreement and precision between all readers and areas was very low. The average percentage of agreement among all readers (for all areas) was 66% with a CV of 58%. The results obtained for the group of 9 expert readers did not improve much (mean agreement of 71.8% and CV of 51.8%). Results varied quite much between areas, but in general the agreement with the modal age was low, being highest for areas 7 and 8 (with 80% agreement in both cases). A group of readers corroborated a difference of criteria (probably linked to a poor distinction between checks and true winter translucent marks) by which tend to assign older ages than the rest of the readers.
- Following the recommendation of WGBIOP Guidelines for Workshops on Age Calibration (ICES, 2015) of formulating species (and stock specific) target and threshold statistics as a tool for the evaluation of the quality of age readings, and taking into account the results for the Bay of Biscay anchovy (where several workshops and exchanges have regularly taken place and age validations are achieved), WKARA2 suggest threshold values of agreements of 80% and of max CVs of 20% across ages, and targets for agreements around 90%, or more, and CV around 10% or less.
- The starting position at the beginning of the workshop was that growth patterns of otolith vary quite much between different areas. This was presumed according to the relevant discrepancies in age determination between readers for the different areas under study. However as we progressed on the discussions and presentations during the workshop it became evident that the differences may not be so relevant as initially believed, and that a common general growth pattern could emerge for all areas (with some exceptions).
- Major differences may appear however on the intensity of the winter hyaline zones, so that the more to the North an area is placed, the greater the intensity of winter translucent zones (Bay of Biscay, Gulf of Lion, Adriatic). The most southern regions seem to lay down less pronounced winter hyaline zones, showing less contrast between hyaline and opaque zones, and therefore increasing the difficulties for identifying true winter rings from other hyaline marks or checks appearing quite often in these otoliths (Morocco, Tunis, Sicily). Finally Gulf of Cadiz, Ionian, Southern Tyrrhenian and Aegean seas seem to show an intermediate intensity of hyaline marking. Checks have been admitted and identified in all the areas examined in WKARA2.
- Following the workshop discussions there has been a progressive change in the perception of the growth pattern applicable to these anchovy otoliths in

many areas by which growth at ages 0 and 1 are far prominent than at older ages and the occurrence of checks was more frequently admitted. Typically annulus width during the first, second and third year of life (corresponding to 0, 1 and 2-years-old groups) decreases progressively. Oldest ages (3 and 4) present a more similar widths (but still slightly decreasing) compared to the one experienced at previous ages. Most typical checks appear at ages 0 (C05, C08) and 1 (C12, C15) where occasionally multiple rings and/or spawning check can occur. This led to some revisions of the otolith interpretation and assigned ages to a substantial amount of otoliths.

- Major difficulties appearing in the age assignation arise from:
 - o Differentiating between true annual winter hyaline rings and false rings (or checks), mainly the first annual winter ring.
 - o Recognizing weakly formed winter rings which masks the general pattern of growth.
 - o Insufficient typical annual growth pattern recognition and insufficient criteria regarding on the otolith edge that can be expected to be seen along the year
 - o Recognizing the opaque or hyaline nature of the edge which may affect the age determination.
- There have been evidences that the age determination rule for the birthdate at the middle of the year has in some instances been inconsistently applied, particularly in the first half of the year. This was probably due to the complication of the changing rule for age assignation for the two halves of the year (prior and post birthdate). For assessment models running on natural calendar year it might be better (simpler) to assume birthdates on 1 Jan.
- These evidences lead to conclude on the need to review past age determinations. Although this task could be delayed until the 2018 exchange is done, in order to be sure that all the readers apply the protocol and the current criteria of this workshop coherently, since current criteria would change the otoliths interpretation and the age determination in many areas.
- Finally, this Workshop adopted a common protocol for all areas in order to standardize the anchovy age assignments and to improve the age estimates. In addition the age assignation rules for the two halves of the year have been rewritten to assure a better understanding of their inherent logic.
- An agreed collection of otoliths by areas were produced and upload to the Age Reader's Forum. The otoliths that are part of the collection have been taken into account those in which the percentage of agreement among expert readers was over 80% in the 2014 Exchange.

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Annex 1: WKARA2 agenda

AGENDA of WORK

	Monday, November 28, 2016
10:30 – 11:30	Opening of the meeting; presentation of the agenda and participants; local and network arrangements; brief overview of ToRs and report content
11:30 – 13:45	Review information on age estimations, otolith exchanges, workshops and validation work done so far (<i>ToRa</i>) and beginning of the validation studies by regions: <i>Presentations of the Review information and Anchovy Bay of Biscay validation.</i>
13:45-15:30	<i>Lunch break</i>
15:30 – 16:30	Presentation of Validation studies and growth increment patterns of the different areas (<i>ToRa and b</i>): <i>Studies presentations of anchovy from IXa North and IXa South.</i>
16:30 – 16:45	<i>Coffee break</i>
16:45 – 18:30	Presentation of Validation studies and growth increment patterns of the different areas (<i>ToRa and b</i>): <i>Studies presentations of Anchovy from Alboran Sea (GSA01), Western Mediterranean (GSA06) and Strait of Sicily (GSA 16).</i>

	Tuesday, November 29 , 2016
09:00 – 11:00	Presentation of Validation studies and growth increment patterns of the different areas (<i>ToRa and b</i>): <i>Studies presentations of anchovy from Aegean Sea (GSA 20 & GSA22) and Tunisian waters (GSA 12, 13 and 14)</i>
11:00 – 11:15	<i>Coffee break</i>
11:15 – 11:45	Presentation and discussion of the 2014 otolith exchanges results, comparison of precision against modal age and bias; evaluation of levels of agreement among readers and institutes (<i>ToRs a and c</i>): <i>Exchange results Presentation</i>
11:45 – 13.45	Identification of problems and difficulties in age estimation of anchovy, including on-screen discussion of relevant otolith readings from the exchange. (<i>ToR c</i>): <i>Presentation of Exchange Otoliths from VII and VIII areas</i>
13:45– 15:00	<i>Lunch break</i>

15:00 – 16:30	Identification of problems and difficulties in age estimation of anchovy, including on-screen discussion of relevant otolith readings from the exchange. (ToR c): <i>Presentation of Exchange Otoliths from Division IXa and Alboran Sea</i>
16:30-16:45	<i>Coffee break</i>
16:45-18.30	Identification of problems and difficulties in age estimation of anchovy, including on-screen discussion of relevant otolith readings from the exchange. (ToR c): <i>Presentation of Exchange Otoliths from Western Mediterranean and Gulf of Lion</i>

	Wednesday, November 30, 2016
09:00 – 11:00	Identification of problems and difficulties in age estimation of anchovy, including on-screen discussion of relevant otolith readings from the exchange. (ToR c): <i>Presentation of Exchange Otoliths from Southern Tyrrhenian, Strait of Sicily, Ionian Sea and Aegean Sea</i>
11:00 – 11:15	<i>Coffee break</i>
11:15 – 13:00	<p>Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation (based on exchange otoliths where age structures have been identified by all readers) – this will lead to collation of a set of agreed-age otoliths for a reference collection (ToR b, c and e).</p> <p>Age readers also bring other images of otoliths of typical and easy read otoliths and of other with difficulties on age determination, to analyse and discuss in the group: <i>Presentation of Otoliths from Bay of Biscay (8.cb) and IXa north by readers area</i></p>
13:45 – 15:30	<i>Lunch break</i>
15:30 – 17:15	<p>Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation (based on exchange otoliths where age structures have been identified by all readers) – this will lead to collation of a set of agreed-age otoliths for a reference collection (ToR b, c and e).</p> <p>Age readers also bring other images of otoliths of typical and easy read otoliths and of other with difficulties on age determination, to analyse and discuss in the group: <i>Presentation of Otoliths from Gulf of Cadiz (IXa South), Alboran Sea (GSA01) and Western Mediterranean (GSA06) by readers area.</i></p>

17:15 – 17:30	<i>Coffee break</i>
17:30 – 18:30	<p>Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation (based on exchange otoliths where age structures have been identified by all readers) – this will lead to collation of a set of agreed-age otoliths for a reference collection (<i>ToR b, c and e</i>).</p> <p>Age readers also bring other images of otoliths of typical and easy read otoliths and of other with difficulties on age determination, to analyse and discuss in the group: <i>Presentation of Otoliths from Morocco area by reader area</i></p>

	Thursday, December 1, 2016
09:00 – 11:00	<p>Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation (based on exchange otoliths where age structures have been identified by all readers) – this will lead to collation of a set of agreed-age otoliths for a reference collection (<i>ToR b, c and e</i>).</p> <p>Age readers also bring other images of otoliths of typical and easy read otoliths and of other with difficulties on age determination, to analyse and discuss in the group: <i>Presentation of Otoliths from Adriatic Sea (GSA 17) and Tunisian waters (GSA 12,13 and 14) by readers area</i></p>
11:00 -11:15	<i>Coffee break</i>
11:15 – 13:30	<p>Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation (based on exchange otoliths where age structures have been identified by all readers) – this will lead to collation of a set of agreed-age otoliths for a reference collection (<i>ToR b, c and e</i>).</p> <p>Age readers also bring other images of otoliths of typical and easy read otoliths and of other with difficulties on age determination, to analyse and discuss in the group: <i>Presentation of Otoliths from Ionian Sea (GSA 20), Aegean Sea (GSA 22) and from Strait of Sicily (GSA 16) by readers area</i></p>
13:30 – 15:00	<i>Lunch</i>

15:00 – 16:30	Update age reading criteria based on the validation studies results, growth increment pattern and the discussion of relevant otolith readings from the exchange and from different laboratories (<i>ToR b</i>).
16:30 – 16:45	<i>Coffee break</i>
16:15 – 18: 00	Development of a common manual for age determination of anchovy (writing, plotting, discussing, etc). (<i>ToR b</i>).

	Friday, December 2, 2016
9:00 – 11:00	Recommendations based on the Workshop results and draft report elaboration
11:00 -11:15	<i>Coffee break</i>
11:15 – 13:00	Draft report elaboration.
13:00	Time for goodbyes and bon voyage's...

Annex 2: List of participants

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Annex 3: Recommendations

Recommendation	For follow up by:
1. WKARA2 recommends to carry out validation studies on age determination for the different areas inhabited by the anchovy populations either via micro-increment preparation (at least to validate the first annulus for each area) or by other methods as studies of progression of length frequency modes throughout time, for tracking cohorts, etc.	WGBIOP, WGHANSA,
2. WKARA2 recommends to review the convenience of setting date of birthdate at the middle of the year for anchovies in some Mediterranean areas and to consider to move to 1st January, because of the difficulties perceived during the workshop on the application of a changing rule for the first and second halves of the year (as associated to birthdate 1 July) and for simplicity and coherence in naming age classes in correspondence with the year classes used in the assessments based on natural calendar year (Jan-Dec).	WGBIOP
3. WKARA2 recommends, as far as possible, that only the age readings of the most expert readers are used for the assessment inputs and second that new readers pass a training processes from validated set of otoliths of the area they have to work with.	WGBIOP, WGHANSA
4. WKARA2 recommends the realization of a small exchange to be carried out during 2018 in order to see if the update Age reading protocol have been adopted by all readers (at least the participants in WKARA2) and to see if the accuracy and precision has improved.	WGBIOP, WGHANSA
5. WKARA2 recommends the realization of the intercalibration exercises by areas (for the different countries taking part in ageing reading on the same stocks). This becomes compulsory for regions where several countries exploit the same stock.	WGBIOP, WGHANSA
6. WKARA2 recommends that all age readers who participate in an exchange should also participate (preferably attending physically) in the subsequent workshops.	WGBIOP

Annex 4: Detailed results of 2014 otolith exchange

Annex 4.1 ANE_VII- English Channel

Table: Age reading results by reader

Table 1		Anchovy Otolith SET VII-English Channel (Anchovy Exchange 2014)																								Range r.1-6				
		Sample		Pen	Pen	Sex	Landing	Sp AU	Sp IR	Fr PG	IR SG	Sp PT	Sp OD	Sp CN	Sp JT	IR PC	IR LC	Sp AA	IR SM	IR MP	Gr CM	Fr EB	Po ES	Gr DP	Si TM	IR PG	MODAL	Percent	Precision	
Status	Year	ID	no	length			month	Reader1	Reader2	Reader3	Reader4	Reader5	Reader6	Reader7	Reader8	Reader9	Reader10	Reader11	Reader12	Reader13	Reader14	Reader15	Reader16	Reader17	Reader18	Reader19	age	agreement	CV	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0081.jpg	-	95.0	M	10		0	0	0	0	0	0	-	0	0	1	0	0	0	0	0	0	0	1	0	98%	291%		
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0082.jpg	-	100.0	F	10		0	0	0	0	0	0	-	0	0	1	0	0	0	0	0	0	0	1	0	98%	291%		
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0045.jpg	-	106.0	F	10		0	0	0	0	0	0	-	0	0	1	0	1	0	0	0	0	0	0	1	0	83%	230%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0041.jpg	-	110.0	M	10		0	0	0	0	0	0	-	0	0	1	0	1	0	0	0	1	0	0	2	0	78%	207%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0094.jpg	-	116.0	F	10		0	0	0	1	0	1	-	0	0	1	1	1	0	1	0	1	1	2	2	0	44%	103%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0063.jpg	-	120.0	F	10		0	0	0	0	0	0	-	0	0	1	0	1	0	0	0	0	0	0	2	0	83%	247%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0066.jpg	-	125.0	F	10		0	0	0	0	0	0	-	0	0	1	0	1	0	0	0	0	0	0	2	0	83%	247%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0091.jpg	-	130.0	F	10		0	0	0	0	0	0	-	0	0	1	0	1	1	0	0	0	0	0	1	2	0	72%	178%
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0082.jpg	-	136.0	F	10		0	0	0	0	0	0	-	0	0	1	0	1	1	0	0	0	0	1	2	0	72%	178%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0066.jpg	-	145.0	F	10		0	0	0	1	0	0	-	0	0	1	0	1	1	0	0	0	1	0	3	0	67%	176%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0014.jpg	-	155.0	M	9		1	2	2	2	2	2	-	2	1	3	2	2	2	2	3	2	3	3	2	61%	31%		
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0011.jpg	-	160.0	M	9		1	1	2	2	1	1	-	1	1	2	1	2	1	1	2	2	1	2	3	1	61%	43%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0005.jpg	-	165.0	M	9		1	1	1	2	1	1	-	1	1	2	1	2	2	2	3	2	2	2	3	1	44%	41%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0001.jpg	-	170.0	M	9		2	2	1	2	2	2	-	2	1	2	2	2	2	2	2	2	2	2	4	2	83%	30%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0008.jpg	-	178.0	F	9		1	1	1	1	1	1	-	1	1	2	1	2	2	1	2	1	1	4	4	1	67%	63%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0029.jpg	-	190.0	F	9		1	1	1	3	2	2	-	1	1	3	2	2	3	2	3	3	3	4	4	2	39%	43%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0037.jpg	-	188.0	M	9		1	3	1	2	2	3	-	2	1	3	3	3	3	2	3	3	2	2	4	3	44%	36%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0038.jpg	-	190.0	M	9		1	2	1	3	2	2	-	1	2	3	2	2	2	2	-	2	2	3	4	2	59%	37%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0035.jpg	-	196.0	M	9		2	2	1	2	2	2	-	2	2	4	2	2	3	2	4	2	2	4	6	2	67%	42%	
Sem 2	2014	JC_14_TRIM3_OAMANOC_O_0036.jpg	-	200.0	F	9		2	2	1	3	2	2	-	2	-	3	2	3	3	2	4	4	2	4	6	2	47%	39%	
Total read								20	20	20	20	20	20	0	20	19	20	20	20	20	20	19	20	20	20	20				
Total NOT read								0	0	0	0	0	0	20	0	1	0	0	0	0	0	0	1	0	0	0	0		66.7%	127.6%

Table: ANE_VII- English Channel. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	10	76.1%	214.8%	0.29
1	3	57.4%	49.0%	0.56
2	6	59.4%	36.8%	0.25
3	1	44.4%	-	-0.61
4	-	-	-	-
5	-	-	-	-
Total	20	66.7%	127.6%	0.27
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	8	65.3%	158.3%	0.42
1	2	55.6%	96.3%	-0.17
2	8	60.6%	35.7%	0.23
3	2	50.0%	27.3%	0.00
4	-	-	-	-
5	-	-	-	-
Total	20	60.9%	90.0%	0.24
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	10	93.3%	106.3%	0.08
1	4	75.0%	49.8%	0.36
2	6	62.3%	36.0%	-0.04
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	20	80.4%	73.9%	0.10

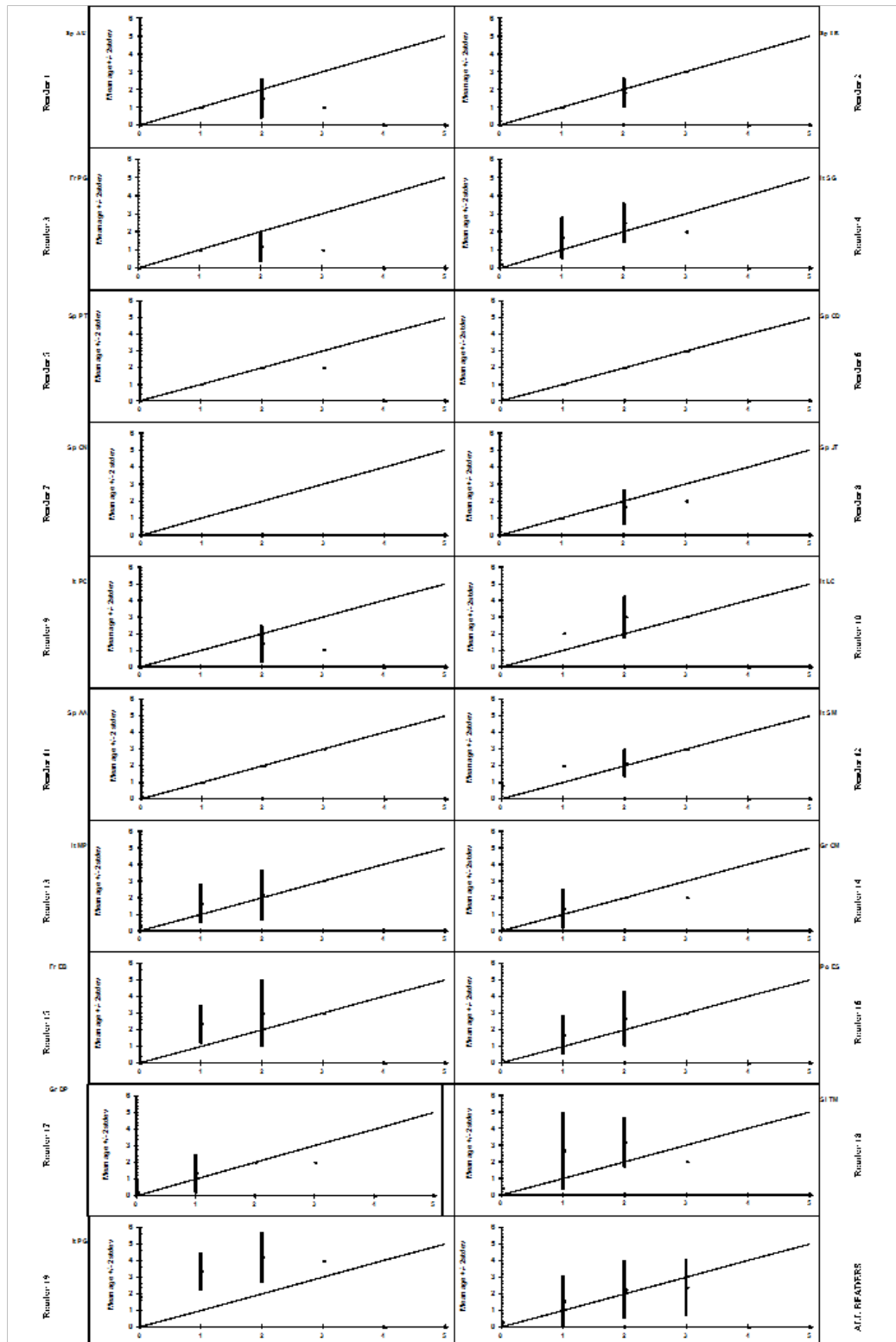


Figure: ANE_VII-English Channel. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

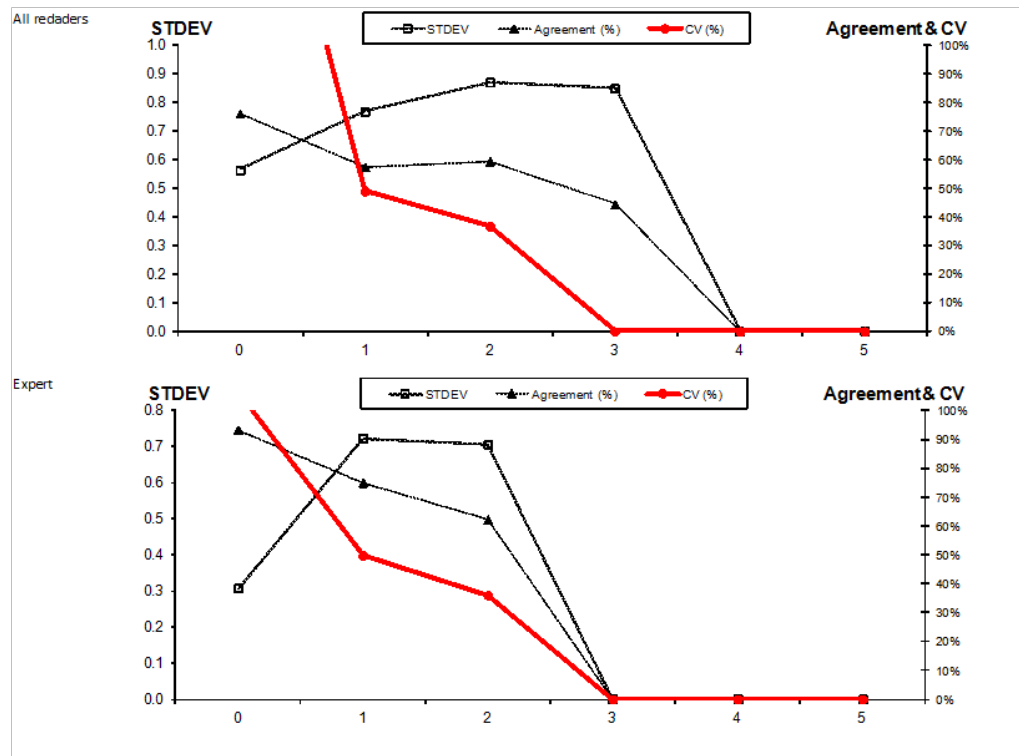


Figure: ANE_VII- English Channel. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age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

Table: Age reading results by reader

Table 1		Anchovy Otolith SE I VIII-Bay of Biscay (Anchovy Exchange 2014)																									RANGE ± 1 SE			
Stratum	Year	Sample no	Fish no	Fish length	Sex	Landing month	Sr AU	Sr IR	Px PB	Sr BG	Sr PT	Sr CD	Sr ON7	Sr UT	Sr PO	Sr LO	Sr AK	Sr EM	Sr MP	Sr OM	Px EB	Px ES	Sr DP	Sr TM	Sr PO	Sr AG	Percent mortal	Growth CV		
Sem 1	2013	ANE08032013_91_01.jpg	-	104.0	female	3	1	1	1	0	4	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	69%	123%	
Sem 1	2013	ANE08032013_78_02.jpg	-	114.0	male	3	2	2	2	1	1	1	1	-	0	2	1	1	1	1	1	-	0	1	1	2	1	69%	84%	
Sem 1	2013	ANE19082013_83_03.jpg	-	128.0	male	6	1	2	1	1	1	-	-	1	1	1	1	1	1	1	1	-	0	1	2	1	1	76%	43%	
Sem 1	2013	ANE19082013_04_04.jpg	-	118.0	male	6	2	2	1	1	1	-	-	1	1	1	1	1	1	1	1	2	2	2	2	2	2	74%	24%	
Sem 1	2013	ANE21032013_80_06.jpg	-	166.0	male	1	1	2	1	1	1	1	-	1	2	2	1	1	1	1	1	-	1	1	3	2	1	71%	48%	
Sem 1	2013	ANE21032013_89_06.jpg	-	142.0	male	3	1	1	1	1	1	2	-	1	2	1	1	1	0	1	-	-	1	1	2	3	1	66%	62%	
Sem 1	2013	ANE21032013_49_07.jpg	-	167.0	female	3	1	2	1	2	1	2	-	1	2	2	1	1	1	1	1	-	1	1	2	2	1	69%	26%	
Sem 1	2013	ANE19082013_10_08.jpg	-	160.0	female	6	1	1	1	1	1	-	-	1	1	1	1	1	1	1	1	-	1	1	1	1	1	69%	20%	
Sem 1	2013	ANE21032013_36_09.jpg	-	149.0	male	3	1	1	1	2	1	1	-	1	2	1	1	1	1	1	1	-	1	1	2	2	1	76%	36%	
Sem 1	2013	ANE06032013_114_10.jpg	-	170.0	female	3	3	2	2	2	2	2	-	1	2	3	2	1	2	1	3	2	2	3	3	3	2	67%	25%	
Sem 2	2011	ANIE112011_12_5_11.jpg	-	35.0	undefined	11	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0%	0%	
Sem 2	2011	ANIE0112011_114_E_12.jpg	-	114.0	undefined	11	0	0	0	0	0	0	-	0	0	1	0	0	0	0	-	0	0	0	0	1	0	68%	180%	
Sem 2	2013	ANE03122013_119_13.jpg	-	127.0	female	12	1	1	1	0	1	1	-	1	1	1	1	1	1	1	1	-	1	2	1	2	1	62%	40%	
Sem 2	2013	ANE02092013_16_14.jpg	-	128.0	female	9	1	2	1	1	1	1	-	1	2	1	1	1	1	1	1	1	1	1	2	3	1	78%	45%	
Sem 2	2013	ANE08082013_10_15.jpg	-	137.0	male	8	1	1	1	1	1	1	-	1	2	1	1	1	1	1	1	1	1	1	1	1	1	84%	20%	
Sem 2	2013	ANE08082013_20_16.jpg	-	146.0	male	8	2	2	2	2	2	1	-	1	3	2	2	1	1	2	2	2	2	2	3	2	1	63%	33%	
Sem 2	2013	ANE08082013_19_17.jpg	-	166.0	male	8	2	2	2	3	3	3	-	2	3	3	1	1	1	3	2	2	3	3	3	3	0	61%	20%	
Sem 2	2013	ANE01122013_20_18.jpg	-	166.0	male	12	1	1	1	1	1	1	-	1	2	2	1	1	1	1	2	2	2	2	2	2	2	62%	40%	
Sem 2	2011	ANIE0112011_115_E_19.jpg	-	175.0	female	11	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	2	3	2	69%	17%	
Sem 2	2011	ANIE0112011_115_E_21_20.jpg	-	183.0	male	11	2	1	1	2	2	1	-	1	2	2	1	1	2	2	2	-	1	2	2	4	2	63%	48%	
Sem 1	2013	RE0206.jpg	-	136.0	male	3	1	1	1	1	1	-	-	1	-	1	1	1	2	1	1	1	1	1	2	1	68%	20%		
Sem 1	2013	RE0206.jpg	-	136.0	male	3	1	1	1	1	1	-	-	1	-	1	1	1	1	1	1	1	1	1	1	1	1	68%	23%	
Sem 1	2013	RE0206.jpg	-	160.0	male	3	3	3	2	2	2	3	-	2	3	2	2	3	2	2	2	3	2	2	2	3	2	66%	21%	
Sem 1	2013	RE0206.jpg	-	160.0	female	3	1	1	1	2	2	2	-	2	3	2	2	2	2	2	2	2	1	2	2	3	2	67%	31%	
Sem 1	2013	RE0206.jpg	-	155.0	male	3	3	3	3	3	3	3	-	3	3	3	3	3	3	3	3	3	3	3	3	3	1	64%	18%	
Sem 1	2013	RE0206.jpg	-	155.0	female	3	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	3	2	69%	16%	
Sem 1	2013	RE0206.jpg	-	155.0	male	3	3	3	3	3	3	3	-	3	3	2	3	2	2	2	2	2	3	3	2	3	2	67%	18%	
Sem 1	2013	RE0206.jpg	-	166.0	female	3	3	2	2	3	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	2	2	63%	18%	
Sem 1	2013	RE0206.jpg	-	170.0	female	3	3	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	3	2	72%	26%	
Sem 1	2013	ANE-230413-1_02.jpg	-	149.0	male	4	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	2	94%	11%		
Sem 1	2013	ANE-230413-1_03.jpg	-	160.0	male	4	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	2	90%	68%		
Sem 1	2013	ANE-230413-07.jpg	-	149.0	male	4	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	61%	43%	
Sem 1	2013	ANE-230413-09.jpg	-	166.0	male	4	3	3	3	3	3	2	3	-	2	3	2	3	2	2	2	2	2	2	3	3	0	61%	19%	
Sem 1	2013	ANE-230413-10.jpg	-	146.0	male	4	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	3	2	69%	15%		
Sem 1	2013	ANE-230413-11.jpg	-	162.0	male	4	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	67%	6%	
Sem 1	2013	ANE-230413-12.jpg	-	174.0	female	4	2	2	2	2	2	2	-	2	2	4	2	2	2	2	2	2	2	2	4	2	76%	33%		
Sem 1	2013	ANE-230413-13.jpg	-	166.0	female	4	1	1	1	1	1	1	-	1	1	2	1	1	1	2	3	3	3	3	1	66%	48%			
Sem 1	2013	ANE-230413-14.jpg	-	160.0	female	4	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	94%	11%	
Sem 1	2013	ANE-230413-18.jpg	-	180.0	female	4	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	94%	11%	
Sem 1	2013	ANE-230413-36.jpg	-	189.0	female	4	2	3	3	3	3	3	-	3	3	2	3	2	3	2	3	3	2	3	2	3	0	61%	19%	
Sem 1	2013	ANE-230413-39.jpg	-	164.0	female	4	3	3	3	3	3	3	3	-	2	3	3	2	2	2	2	2	3	3	3	3	0	72%	17%	
Sem 1	2013	ANE-230413-43.jpg	-	164.0	female	4	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	67%	6%	
Sem 1	2013	ANE-230413-44.jpg	-	164.0	female	4	3	3	3	2	3	3	-	3	3	2	3	2	2	2	3	3	3	3	3	3	0	61%	19%	
Sem 1	2013	ANE-230413-47.jpg	-	160.0	female	4	3	3	3	3	3	3	3	-	3	3	3	3	3	2	2	2	3	3	3	3	0	63%	14%	
Sem 2	2010	JUV10-9024_17.jpg	-	91.0	undefined	9	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	1	0	69%	291%		
Sem 2	2010	JUV10-9024_19.jpg	-	84.0	undefined	9	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	1	0	64%	24%	
Sem 2	2010	JUV10-9024_20.jpg	-	84.0	undefined	9	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100%	0%	
Sem 2	2013	ANE-260913-1_01.jpg	-	158.0	undefined	9	1	1	1	1	1	1	-	1	1	3	1	2	3	1	1	1	2	1	4	1	67%	61%		
Sem 2	2013	ANE-260913-03.jpg	-	162.0	undefined	9	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	65%	45%	
Sem 2	2013	ANE-260913-04.jpg	-	166.0	male	9	2	2	2	2	2	2	-	1	3	2	2	2	2	2	2	2	2	2	3	2	63%	20%		
Sem 2	2013	ANE-260913-05.jpg	-	169.0	undefined	9	1	1	1	2	1	1	-	1	2	3	1	2	2	1	1	1	1	1	4	1	61%	66%		
Sem 2	2013	ANE-260913-06.jpg	-	166.0	undefined	9	1	1	1	2	2	1	-	1	2	2	1	2	1	1	1	1	1	2	3	1	66%	41%		
Sem 2	2013	ANE-260913-07.jpg	-	163.0	undefined	9	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	2	3	1	67%	40%	
Sem 2	2013	ANE-260913-08.jpg	-	166.0	male	9	2	2	2	2	2	2	-	1	2	2	2	2	2	2	2	2	2	1	3	2	63%	21%		
Sem 2	2013	ANE-260913-15.jpg	-	166.0	female	9	2	2	2	2	2	1	-	1	3	2	2	2	2	2	2	2	2	2	2	3	2	76%	24%	
Sem 2	2013	ANE-260913-16.jpg	-	166.0	female	9	1	1	1	1	1	1	-	1	2	2	1	2	2	2	2	1	1	1	1	3	2	69%	44%	
Sem 2	2013	ANE-260913-16.jpg	-	164.0	female	9	1	1	1	1	1	1	-	1	2	2	1	2	2	1	1	1	1	1	2	1	1	67%	41%	
Sem 2	2013	ANE-260913-17.jpg	-	163.0	undefined	9	3	3	3	2	2	2	-	1	3	3	2	2	2	2	2	2	2	2	3	2	67%	26%		
Sem 2	2013	ANE-260913-18.jpg	-	161.0	male	9	1	1	1	1	1	1	-	1	1	2	1	1	1	1	1	1	1	1	3	1	69%	44%		
Sem 2	2013	ANE-260913-18.jpg	-	160.0	undefined	9	1	1	1	1	1	1	-	1	1															

Table: ANE_VIII- Bay of Biscay. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	88.5%	186.8%	0.12
1	28	71.3%	44.3%	0.33
2	25	78.5%	23.1%	0.05
3	11	65.2%	19.9%	-0.33
4	-	-	-	-
5	-	-	-	-
Total	70	74.3%	45.1%	0.11
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	86.3%	145%	0.14
1	24	70.3%	49%	0.36
2	34	71.6%	26%	0.15
3	6	59.3%	24%	-0.37
4	-	-	-	-
5	-	-	-	-
Total	70	71.3%	44%	0.17
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	5	100.0%	0.0%	0.00
1	27	80.8%	32.8%	0.18
2	26	79.1%	19.2%	-0.06
3	12	76.9%	15.2%	-0.24
4	-	-	-	-
5	-	-	-	-
Total	70	80.8%	22.4%	0.00
Bay of Biscay readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	5	100.0%	0.0%	0.00
1	31	89.2%	17.5%	0.11
2	22	91.6%	7.9%	0.01
3	12	90.3%	6.8%	-0.10
4	-	-	-	-
5	-	-	-	-
Total	70	90.9%	11.4%	0.03

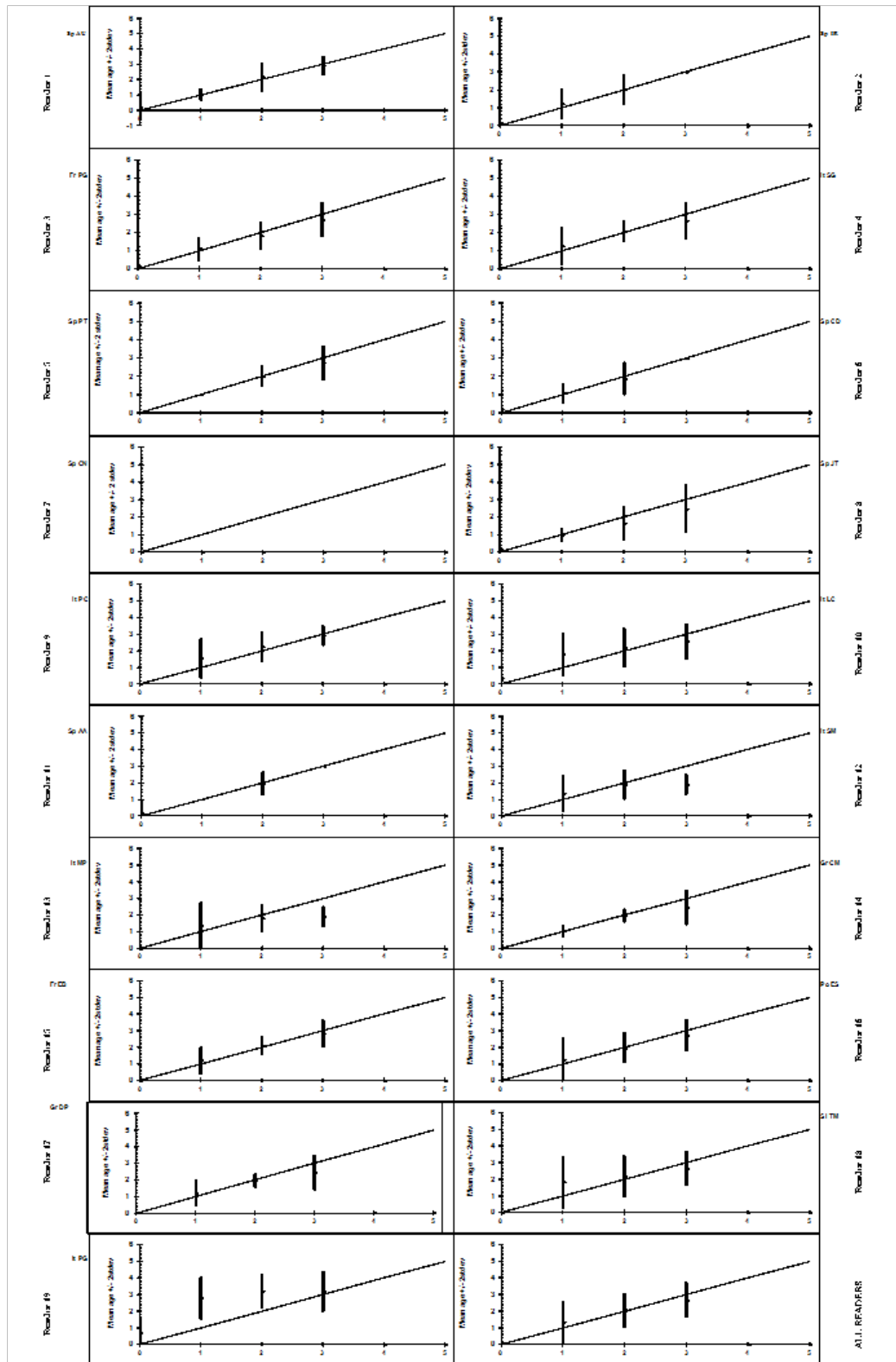


Figure: ANE_VIII- Bay of Biscay. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

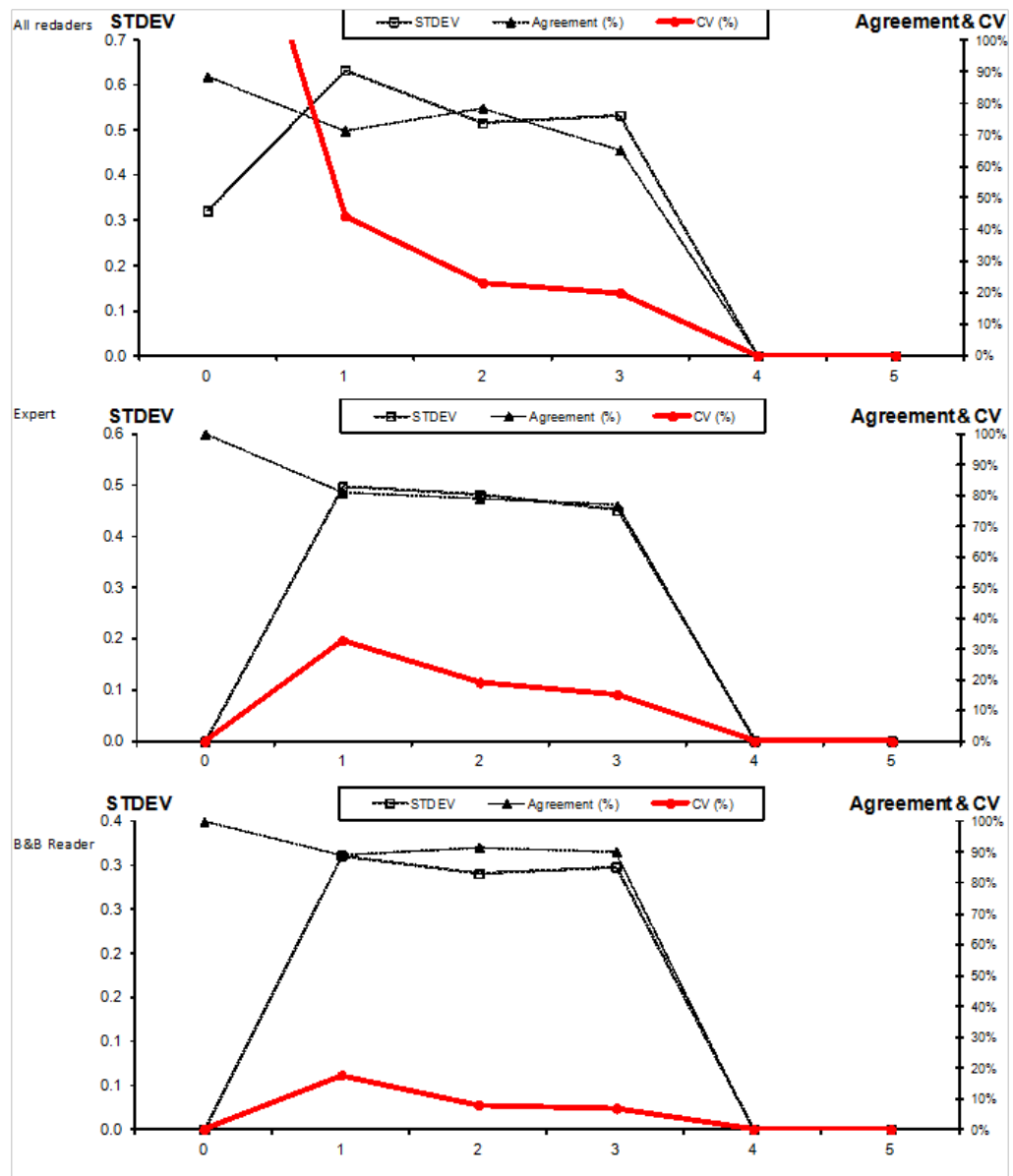


Figure: ANE_VIII- Bay of Biscay. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age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

Table: Age reading results by reader

RANGE
2.4.45

[illegible]

Table: ANE_9a- Gulf of Cadiz and Portugal Coast. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	5	87.8%	203.8%	0.13
1	64	68.6%	43.3%	0.30
2	23	64.1%	31.8%	-0.01
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	92	68.5%	49.1%	0.21
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	5	75.6%	135.5%	0.27
1	47	68.0%	44.0%	0.30
2	39	61.4%	32.7%	-0.03
3	1	55.6%	-	-0.22
4	-	-	-	-
5	-	-	-	-
Total	92	65.4%	43.9%	0.15
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	90.7%	19.8%	0.15
1	71	75.5%	37.9%	0.23
2	15	74.8%	25.5%	-0.07
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	92	76.4%	34.7%	0.18
IXa readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	94.4%	28.9%	0.06
1	51	79.7%	32.9%	0.22
2	35	66.7%	33.8%	-0.22
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	92	75.7%	33.0%	0.04

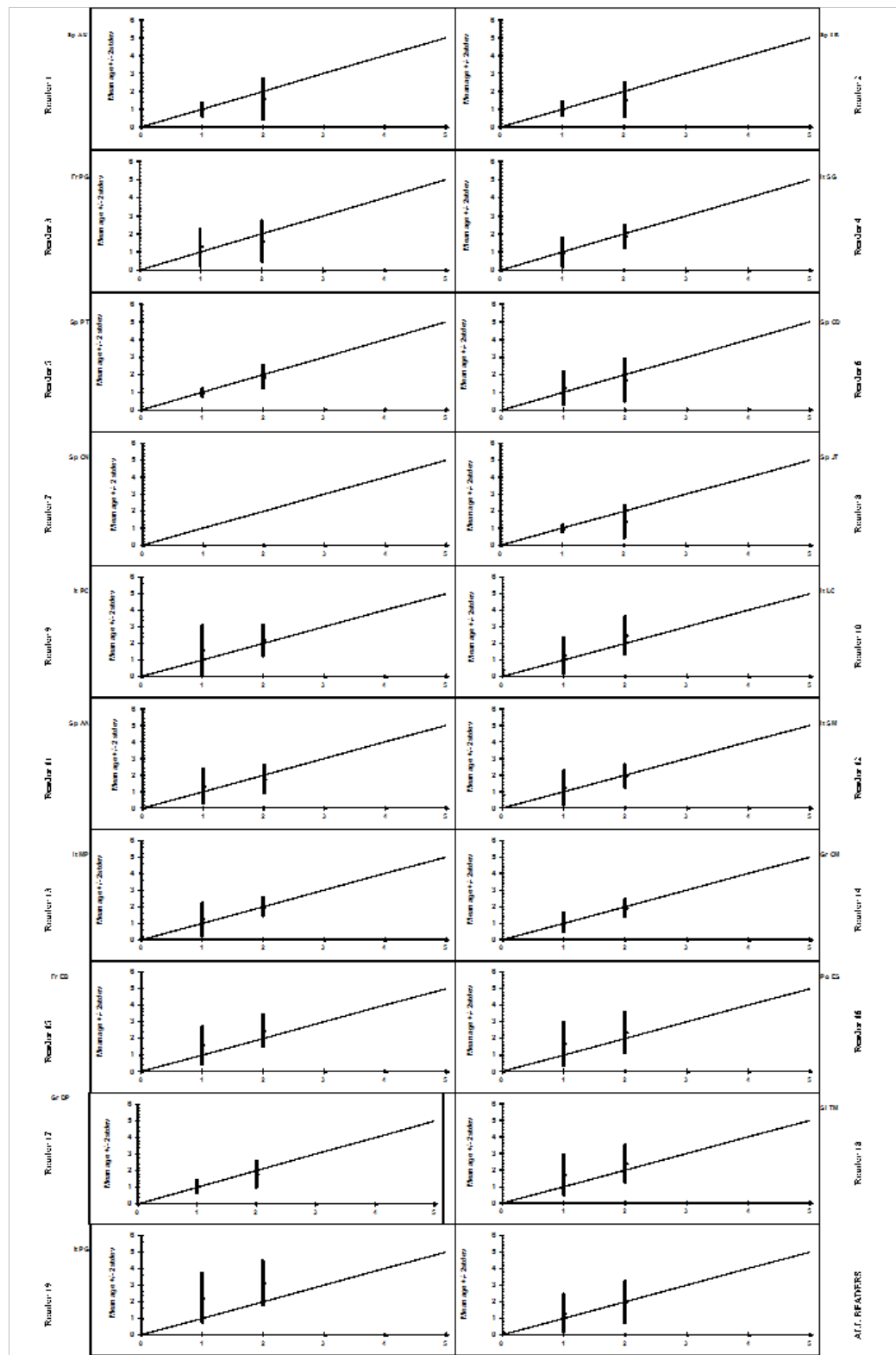


Figure: ANE_IXa- Gulf of Cadiz and Portugal Coast. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

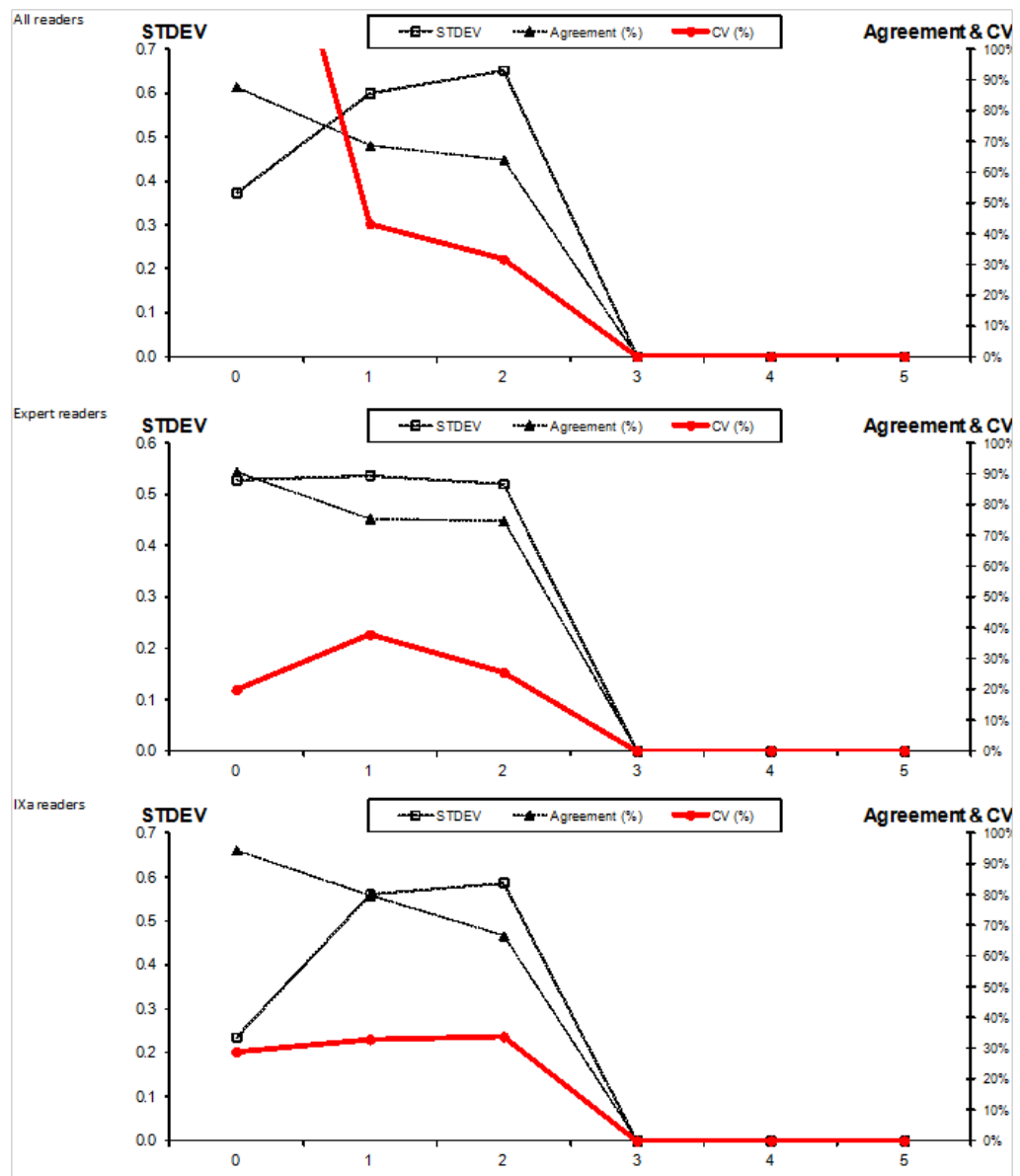


Figure: ANE_9a- Gulf of Cadiz and Portugal Coast. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.4 ANE_GSA01- Alboran Sea

Table: Age reading results by reader

Table 1		Anchovy Otolith SET GSA01_Alboran Sea (Anchovy Exchange 2014)																				RANGE 7.1-16		Percent	Precision			
Sample	Year	Sex	Length	Month	Sp AU	Sp IR	Fr PB	Fr SB	Sp PT	Sp OD	Sp CN	Sp JT	Fr PC	Fr LC	Sp AA	Fr SM	Fr MP	Gr CM	Fr EB	Fr ES	Gr DP	Gr TM	Fr PB	MODAL	Agreement	CV		
Sem 1	2013	02052013-001.jpg	-	142.0	female	5	0	2	3	2	1	-	1	2	2	1	2	2	2	4	3	2	3	3	2	44%	46%	
Sem 1	2013	02052013-002.jpg	-	157.0	female	5	0	1	2	2	1	-	0	2	2	1	2	2	2	3	3	2	2	3	2	50%	52%	
Sem 1	2013	02052013-003.jpg	-	155.0	male	5	0	1	1	2	1	-	0	2	2	1	2	2	2	3	0	2	1	3	2	39%	64%	
Sem 1	2013	02052013-004.jpg	-	148.0	female	5	0	1	1	2	1	-	0	1	2	1	2	2	2	3	1	2	1	3	1	44%	59%	
Sem 1	2013	02052013-005.jpg	-	138.0	male	5	0	1	1	2	0	0	-	0	1	2	1	1	1	3	1	1	1	2	1	56%	75%	
Sem 1	2013	02052013-006.jpg	-	143.0	male	5	0	1	1	2	0	0	-	0	1	-	0	2	2	2	3	0	2	1	3	0	35%	91%
Sem 1	2013	02052013-007.jpg	-	140.0	female	5	0	1	1	1	1	1	-	0	1	2	1	1	1	1	2	2	1	-	3	1	65%	62%
Sem 1	2013	02052013-008.jpg	-	137.0	female	5	0	1	1	2	0	0	-	0	0	1	0	2	1	1	2	1	1	1	2	1	50%	58%
Sem 1	2013	02052013-009.jpg	-	147.0	female	5	0	1	2	1	1	-	0	1	2	1	2	2	2	2	1	2	2	2	1	2	44%	55%
Sem 1	2013	02052013-010.jpg	-	160.0	female	5	1	1	2	2	2	1	-	1	2	3	1	2	2	2	3	4	2	2	4	2	50%	46%
Sem 1	2013	02052013-011.jpg	-	151.0	female	5	0	1	1	2	1	1	-	0	1	3	1	2	2	2	3	2	1	2	3	1	39%	59%
Sem 1	2013	02052013-012.jpg	-	151.0	male	5	0	1	1	2	1	1	-	0	2	2	1	2	2	2	2	2	2	2	3	2	56%	50%
Sem 1	2013	02052013-013.jpg	-	140.0	male	5	0	1	1	2	0	0	-	0	1	2	1	2	2	1	3	2	1	2	3	1	33%	73%
Sem 1	2013	02052013-014.jpg	-	153.0	female	5	0	1	1	1	1	-	0	1	2	1	2	2	2	2	2	1	2	2	3	2	44%	54%
Sem 1	2013	02052013-015.jpg	-	147.0	female	5	0	1	1	1	1	1	-	0	-	2	1	2	2	1	2	1	1	2	1	1	59%	54%
Sem 1	2013	02052013-016.jpg	-	153.0	female	5	0	1	1	2	1	1	-	0	2	3	1	2	2	2	3	3	1	2	2	2	39%	57%
Sem 1	2013	02052013-017.jpg	-	145.0	female	5	1	1	1	2	1	1	-	0	2	2	1	2	2	2	3	2	1	2	2	2	50%	45%
Sem 1	2013	02052013-018.jpg	-	151.0	female	5	0	1	1	1	0	0	-	0	1	1	0	1	1	1	2	2	1	1	2	1	56%	75%
Sem 1	2013	02052013-019.jpg	-	134.0	male	5	0	0	1	1	0	0	-	0	2	1	0	1	1	1	-	1	1	2	2	1	47%	58%
Sem 1	2013	02052013-020.jpg	-	144.0	male	5	0	1	1	2	1	0	-	0	1	2	1	2	2	1	2	2	1	2	2	2	41%	64%
Sem 1	2013	02052013-021.jpg	-	133.0	male	5	0	1	1	2	1	0	-	0	1	1	1	2	2	1	2	1	1	2	2	1	50%	61%
Sem 1	2013	02052013-022.jpg	-	127.0	male	5	0	1	1	1	0	0	-	0	1	-	0	1	1	1	2	2	1	2	2	1	47%	75%
Sem 1	2013	02052013-023.jpg	-	127.0	female	5	0	0	1	1	0	0	-	0	1	1	0	1	1	1	-	1	1	1	2	1	59%	53%
Sem 1	2013	02052013-024.jpg	-	146.0	male	5	0	1	1	2	1	1	-	0	2	2	1	2	2	3	3	1	3	3	1	1	33%	58%
Sem 1	2013	02052013-025.jpg	-	132.0	male	5	0	1	1	1	1	0	-	0	1	1	0	1	1	1	-	2	1	1	2	1	65%	68%
Sem 1	2013	02052013-026.jpg	-	153.0	male	5	1	2	2	2	2	1	-	1	2	3	1	3	3	1	3	4	2	3	3	0	39%	39%
Sem 1	2013	02052013-027.jpg	-	137.0	female	5	0	1	1	2	0	0	-	0	1	1	0	1	1	1	2	1	1	1	2	1	56%	75%
Sem 1	2013	02052013-028.jpg	-	150.0	female	5	2	1	1	2	1	1	-	0	2	2	1	2	2	2	3	2	1	2	-	2	53%	45%
Sem 1	2013	02052013-029.jpg	-	143.0	male	5	0	1	1	2	1	1	-	0	2	2	1	2	2	-	3	1	1	2	3	2	41%	57%
Sem 1	2013	02052013-030.jpg	-	158.0	male	5	2	2	2	2	2	1	-	0	2	4	1	2	2	2	4	3	2	3	3	2	56%	45%
Sem 1	2013	02052013-041.jpg	-	119.0	male	5	0	0	1	1	0	0	-	0	0	1	0	1	1	-	0	1	0	2	0	0	52%	116%
Sem 1	2013	02052013-042.jpg	-	117.0	female	5	0	0	1	0	0	0	-	-	0	1	0	1	1	0	1	0	1	0	2	0	59%	123%
Sem 1	2013	02052013-043.jpg	-	116.0	female	5	0	0	1	1	0	0	-	0	0	1	0	1	0	1	1	0	1	1	0	1	50%	103%
Sem 1	2013	02052013-044.jpg	-	118.0	male	5	0	0	1	1	0	0	-	0	0	1	0	1	2	0	-	0	1	1	2	0	53%	121%
Sem 1	2013	02052013-045.jpg	-	117.0	male	5	0	0	1	0	0	0	-	0	0	1	0	1	1	0	-	0	1	0	1	0	65%	140%
Sem 2	2013	08052013-001.jpg	-	142.0	female	5	1	2	1	2	2	2	-	1	2	2	2	2	2	-	3	1	3	3	2	59%	34%	
Sem 2	2013	08052013-002.jpg	-	154.0	female	5	1	2	1	3	2	2	-	1	3	2	3	2	-	4	2	2	3	4	2	2	35%	39%
Sem 2	2013	08052013-003.jpg	-	129.0	male	5	1	1	1	2	1	1	-	0	1	1	1	1	1	-	1	1	2	3	1	1	75%	54%
Sem 2	2013	08052013-004.jpg	-	162.0	female	5	2	3	2	3	3	2	-	2	4	4	2	2	2	-	5	2	3	4	2	2	53%	35%
Sem 2	2013	08052013-007.jpg	-	119.0	male	5	1	1	0	1	0	1	-	1	1	1	1	1	0	-	1	1	1	3	1	1	75%	70%
Sem 2	2013	08052013-008.jpg	-	143.0	male	5	2	2	2	2	2	2	-	1	2	3	2	2	2	2	-	5	2	3	4	2	65%	39%
Sem 2	2013	08052013-009.jpg	-	130.0	male	5	1	1	1	2	1	1	-	1	1	2	1	1	1	-	4	1	1	3	1	1	75%	62%
Sem 2	2013	08052013-010.jpg	-	142.0	female	5	1	2	1	3	1	2	-	1	2	2	2	2	2	-	3	2	2	3	2	2	55%	34%
Sem 2	2013	08052013-011.jpg	-	146.0	male	5	1	2	1	3	1	2	-	1	2	2	2	2	2	-	3	1	3	4	2	2	47%	43%
Sem 2	2013	08052013-013.jpg	-	137.0	female	5	1	1	1	2	1	1	-	1	1	1	1	1	2	1	-	3	1	2	3	1	71%	50%
Sem 2	2013	08052013-014.jpg	-	149.0	male	5	1	2	1	2	1	2	-	1	2	1	2	2	2	-	2	1	2	3	2	1	59%	34%
Sem 2	2013	08052013-015.jpg	-	162.0	female	5	1	2	2	3	2	2	-	2	2	3	3	2	-	5	2	4	4	2	2	2	53%	39%
Sem 2	2013	08052013-017.jpg	-	113.0	female	5	0	1	1	1	0	1	-	1	1	1	1	1	1	-	2	1	1	2	1	1	75%	50%
Sem 2	2013	08052013-021.jpg	-	149.0	female	5	1	2	2	3	1	2	-	1	2	2	2	2	2	-	3	2	3	3	2	1	59%	32%
Sem 2	2013	08052013-022.jpg	-	132.0	male	5	0	1	0	1	1	1	-	1	1	1	1	1	1	-	2	1	1	2	1	1	75%	50%
Sem 2	2013	08052013-023.jpg	-	137.0	male	5	1	1	0	2	1	1	-	1	1	-	1	2	2	1	-	3	1	1	3	1	63%	59%
Sem 2	2013	08052013-024.jpg	-	145.0	male	5	1	1	1	2	1	1	-	1	2	-	1	2	2	1	-	3	1	2	3	1	56%	47%
Sem 2	2013	08052013-025.jpg	-	134.0	female	5	1	1	0	1	1	1	-	1	2	-	1	1	2	1	-	3	1	1	2	1	65%	60%
Sem 2	2013	08052013-026.jpg	-	127.0	male	5	0	1	0	1	1	1	-	1	1	-	1	1	1	-	1	1	1	2	1	1	47%	47%
Sem 2	2013	08052013-027.jpg	-	122.0	male	5	1																					

Table: ANE_GSA01- Alboran Sea. Summary of the average percentage of agreement, CV and relative bias by age.

All readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	6	52.4%	117.6%	0.60	
1	40	65.5%	58.0%	0.12	
2	23	50.5%	45.5%	-0.11	
3	1	38.9%	-	-0.72	
4	-	-	-	-	
5	-	-	-	-	
Total	70	58.9%	58.7%	0.07	
Intermediate & training readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	-	-	-	-	
1	20	68.1%	53.6%	0.19	
2	25	57.6%	36.5%	0.11	
3	3	46.9%	34.2%	-0.28	
4	-	-	-	-	
5	-	-	-	-	
Total	48	62.5%	45.6%	0.13	
Expert readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	14	58.1%	149.1%	0.51	
1	43	68.2%	54.8%	0.02	
2	13	53.8%	40.9%	-0.18	
3	-	-	-	-	
4	-	-	-	-	
5	-	-	-	-	
Total	70	63.5%	71.1%	0.08	

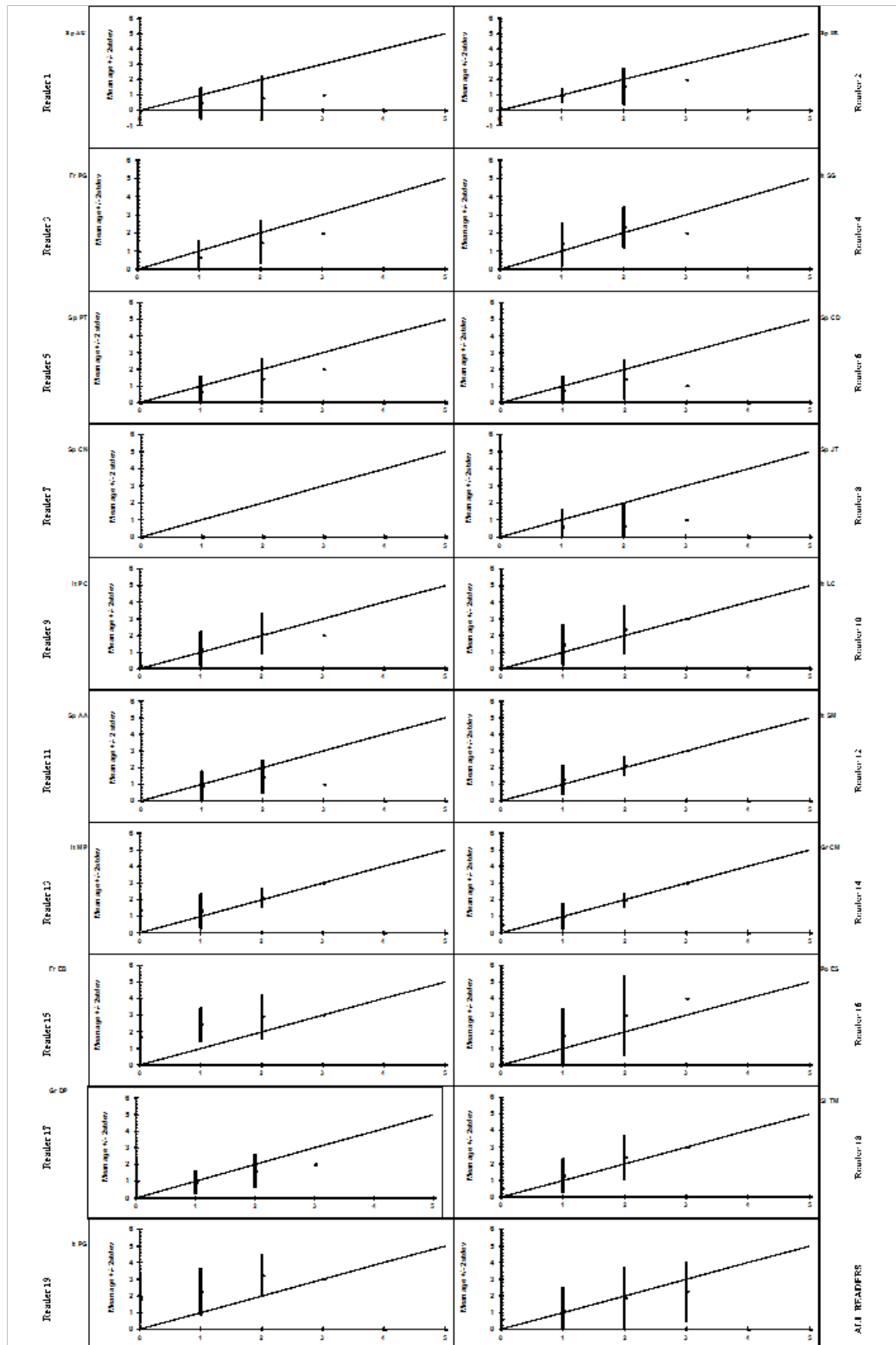


Figure: ANE_GSA01- Alboran Sea. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

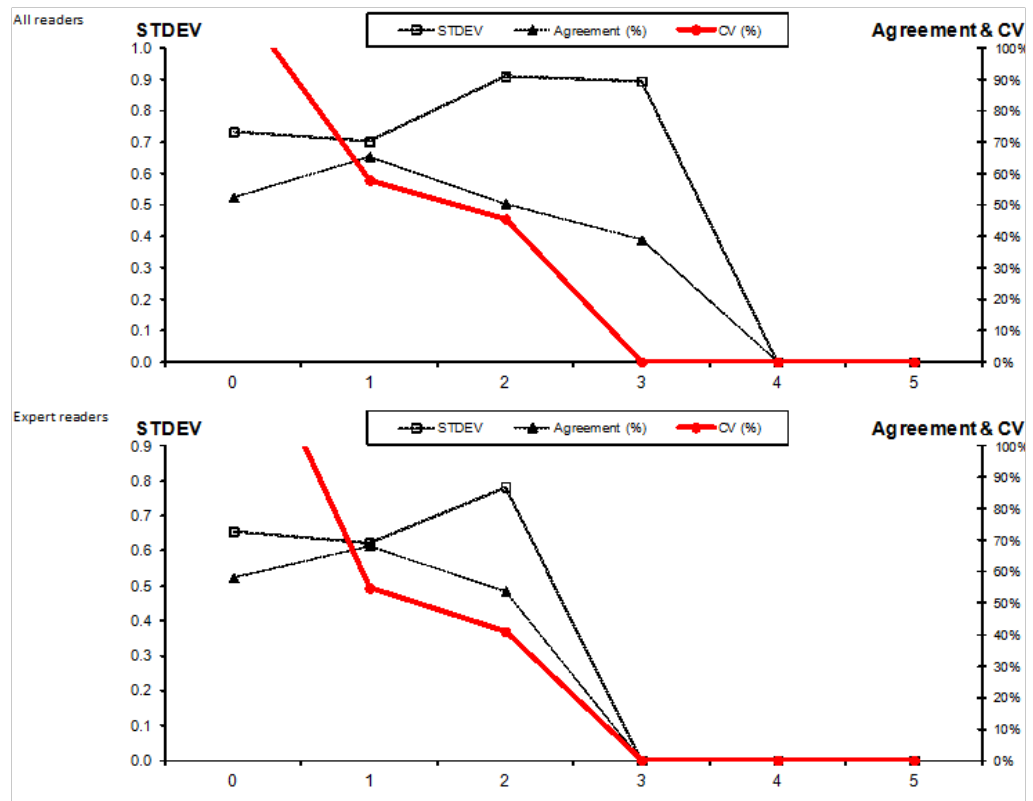


Figure: ANE_GSA01- Alboran Sea. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.5 ANE_GSA06- Western Mediterranean

Table: Age reading results by reader

Table 1		Anchovy Otolith SET GSA06_Western Mediterranean (Anchovy Exchange 2014)																										RANGE t. 1-15			
Stratum	year	Sample no	Flsh no	Flsh length	Sex	Landing month	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	IL SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp CN Reader 7	Sp JT Reader 8	IL PC Reader 9	IL LC Reader 10	Sp AA Reader 11	IL SM Reader 12	IL MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Pu ES Reader 16	Gr DP Reader 17	Sp TM Reader 18	IL PG Reader 19	MODAL Age	Percent	Precision CV			
Sem 1	2013	10042013-001.jpg	-	114.0	male	4	0	0	1	1	0	1	-	0	1	1	1	1	1	1	1	1	2	1	1	2	1	72%	85%		
Sem 1	2013	10042013-002.jpg	-	118.0	female	4	0	0	1	1	0	1	-	0	1	1	1	1	1	1	1	1	2	1	1	2	1	67%	66%		
Sem 1	2013	10042013-004.jpg	-	117.0	female	4	0	0	1	1	0	1	-	0	1	1	1	1	1	1	1	1	3	1	2	1	1	67%	77%		
Sem 1	2013	10042013-006.jpg	-	115.0	female	4	0	0	1	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	2	1	1	67%	70%		
Sem 1	2013	10042013-007.jpg	-	123.0	female	4	1	2	2	1	0	1	-	1	2	1	1	2	2	1	2	1	1	1	2	2	1	50%	44%		
Sem 1	2013	10042013-008.jpg	-	121.0	female	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	1	1	1	1	2	1	76%	55%		
Sem 1	2013	10042013-011.jpg	-	125.0	male	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	1	2	1	1	1	1	76%	55%		
Sem 1	2013	10042013-012.jpg	-	126.0	male	4	1	1	2	0	0	1	-	0	1	-	1	1	1	1	2	1	1	1	1	2	1	65%	61%		
Sem 1	2013	10042013-016.jpg	-	127.0	male	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	-	1	1	1	2	1	1	75%	57%		
Sem 1	2013	10042013-018.jpg	-	131.0	female	4	1	2	1	1	0	1	-	0	2	-	1	1	1	1	1	2	1	1	1	2	1	59%	54%		
Sem 1	2013	10042013-019.jpg	-	132.0	male	4	1	2	3	1	0	1	-	0	2	-	1	1	1	1	2	2	2	1	2	3	1	41%	59%		
Sem 1	2013	10042013-020.jpg	-	130.0	male	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	1	2	1	2	2	1	65%	61%		
Sem 1	2013	10042013-022.jpg	-	135.0	female	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	2	1	2	2	2	1	65%	61%		
Sem 1	2013	10042013-023.jpg	-	135.0	female	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	2	1	2	2	2	1	65%	61%		
Sem 1	2013	10042013-024.jpg	-	135.0	female	4	0	1	1	1	0	1	-	0	1	-	1	1	1	1	1	1	1	1	2	2	1	71%	59%		
Sem 1	2013	10042013-025.jpg	-	136.0	female	4	0	1	2	1	0	1	-	0	1	-	1	1	1	1	1	1	2	1	2	2	1	59%	62%		
Sem 1	2013	10042013-027.jpg	-	140.0	female	4	0	1	1	2	0	1	-	0	-	2	1	2	2	1	1	1	2	2	2	2	1	41%	61%		
Sem 1	2013	10042013-029.jpg	-	142.0	male	4	1	1	1	1	0	1	-	0	1	-	1	2	2	1	1	2	2	1	2	2	1	50%	52%		
Sem 1	2013	10042013-030.jpg	-	142.0	male	4	0	1	1	1	0	1	-	0	1	2	1	2	2	1	1	2	2	2	2	2	1	44%	60%		
Sem 1	2013	10042013-031.jpg	-	150.0	male	4	2	2	3	2	1	1	-	1	2	3	2	2	2	2	2	3	2	2	2	2	2	2	67%	50%	
Sem 1	2013	10042013-032.jpg	-	144.0	female	4	0	1	1	2	0	1	-	0	1	2	1	2	2	1	2	3	2	2	3	2	3	2	39%	64%	
Sem 1	2013	10042013-033.jpg	-	146.0	female	4	1	1	2	2	1	1	-	0	1	2	1	2	2	1	2	2	2	2	2	3	2	50%	45%		
Sem 1	2013	05062013-033.jpg	-	147.0	female	6	0	1	2	1	0	1	-	0	2	2	1	2	2	2	2	2	2	2	2	2	2	2	61%	47%	
Sem 1	2013	05062013-034.jpg	-	149.0	female	6	0	1	1	1	0	1	-	0	1	2	1	2	2	2	1	1	1	1	1	2	1	61%	61%		
Sem 1	2013	05062013-035.jpg	-	145.0	female	6	0	1	2	1	0	1	-	0	2	2	1	2	2	1	1	3	1	1	1	2	1	44%	65%		
Sem 1	2013	05062013-036.jpg	-	147.0	female	6	0	1	1	0	1	1	-	0	1	2	1	2	2	1	1	2	2	1	2	1	2	1	50%	61%	
Sem 1	2013	05062013-037.jpg	-	146.0	female	6	1	2	2	1	1	1	-	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2	61%	40%	
Sem 1	2013	05062013-038.jpg	-	152.0	male	6	0	1	1	1	0	1	-	0	1	3	1	2	2	2	2	2	1	1	2	3	1	50%	70%		
Sem 1	2013	05062013-039.jpg	-	152.0	female	6	0	1	1	1	0	1	-	0	1	3	1	2	2	2	1	1	1	1	2	3	1	56%	72%		
Sem 1	2013	05062013-040.jpg	-	153.0	female	6	0	1	1	1	0	1	-	0	1	2	1	2	2	1	1	1	1	1	3	2	1	56%	67%		
Sem 2	2013	1308013-005.jpg	-	114.0	male	8	1	1	2	1	1	1	-	0	1	0	2	1	1	1	0	2	1	1	2	1	61%	53%			
Sem 2	2013	1308013-007.jpg	-	111.0	male	8	1	1	2	1	1	1	-	1	1	1	1	1	1	1	0	2	1	1	1	2	1	78%	42%		
Sem 2	2013	1308013-008.jpg	-	119.0	female	8	1	1	2	1	1	1	-	1	1	1	1	1	1	1	0	2	1	1	2	2	1	72%	44%		
Sem 2	2013	1308013-010.jpg	-	116.0	male	8	2	1	1	1	1	1	-	1	1	1	1	2	1	1	1	0	2	1	2	2	1	67%	45%		
Sem 2	2013	1308013-013.jpg	-	120.0	female	8	1	1	2	1	1	1	-	1	1	1	1	1	1	1	2	1	1	2	2	2	1	76%	38%		
Sem 2	2013	1308013-015.jpg	-	122.0	female	8	2	1	2	1	1	1	-	1	1	1	1	1	1	1	1	1	2	1	2	3	1	72%	45%		
Sem 2	2013	1308013-016.jpg	-	128.0	female	8	1	2	1	1	1	1	-	1	2	2	1	1	1	1	1	1	2	1	2	3	1	72%	45%		
Sem 2	2013	1308013-017.jpg	-	126.0	female	8	1	2	1	1	1	2	-	1	2	1	1	1	1	1	1	1	2	1	3	3	1	67%	49%		
Sem 2	2013	1308013-018.jpg	-	127.0	female	8	1	2	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	3	1	63%	52%			
Sem 2	2013	1308013-019.jpg	-	126.0	female	8	1	2	1	1	1	1	-	1	-	1	1	1	1	1	0	2	1	1	2	3	1	71%	54%		
Sem 2	2013	1308013-020.jpg	-	130.0	male	8	1	2	1	1	1	2	-	1	1	2	1	1	1	1	0	3	1	1	2	3	1	61%	56%		
Sem 2	2013	1308013-021.jpg	-	130.0	female	8	1	2	1	1	1	2	-	1	2	2	2	1	1	1	1	1	3	1	1	2	1	61%	43%		
Sem 2	2013	1308013-022.jpg	-	132.0	male	8	1	2	1	2	1	2	-	1	2	3	2	2	1	2	2	1	3	2	2	2	2	61%	33%		
Sem 2	2013	1308013-023.jpg	-	134.0	female	8	1	2	1	2	1	2	-	1	2	3	2	2	2	2	2	1	3	2	2	2	2	50%	39%		
Sem 2	2013	1308013-024.jpg	-	136.0	female	8	1	2	1	2	1	2	-	1	0	2	2	2	1	2	1	3	2	2	2	3	2	50%	46%		
Sem 2	2013	1308013-025.jpg	-	137.0	female	8	2	2	2	1	1	2	-	1	2	2	2	2	1	1	2	2	1	3	2	2	3	2	56%	36%	
Sem 2	2013	1308013-026.jpg	-	137.0	male	8	1	2	2	2	1	2	-	1	2	2	2	2	1	2	1	3	2	2	2	3	2	56%	36%		
Sem 2	2013	1308013-027.jpg	-	139.0	female	8	0	2	1	1	1	2	-	1	1	1	1	1	1	1	1	2	1	1	3	1	74%	25%			
Sem 2	2013	1308013-028.jpg	-	140.0	female	8	1	2	1	1	1	2	-	1	1	2	1	1	1	1	1	1	2	1	2	3	1	67%	44%		
Sem 2	2013	1308013-029.jpg	-	140.0	female	8	1	2	1	1	1	2	-	1	2	3	2	2	1	2	1	2	1	3	2	3	3	1	39%	43%	
Sem 2	2013	1308013-030.jpg	-	142.0	male	8	3	2	1	2	1	2	-	1	3	3	2	2	1	2	1	4	2	2	3	3	2	44%	42%		
Sem 2	2013	1308013-031.jpg	-	141.0	female	8	1	2	2	2	1	2	-	1	-	3	2	2	2	2	2	2	3	2	3	3	2	43%	42%		
Sem 2	2013	1308013-032.jpg	-	143.0	male	8	1	2	2	2	1	2	-	1	2	3	2	2	2	2	2	2	3	2	3	4	2	61%	36%		
Sem 2	2013	1308013-033.jpg	-	145.0	female	8	1	2	2																						

Table: ANE_GSA06- Western Mediterranean. Summary of the average percentage of agreement, CV and relative bias by age.

All readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	-	-	-	-	
1	40	62.7%	55.9%	0.17	
2	20	57.4%	38.1%	-0.12	
3	-	-	-	-	
4	-	-	-	-	
5	-	-	-	-	
Total	60	60.9%	49.9%	0.07	
Intermediate & training readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	-	-	-	-	
1	34	70.6%	43.8%	0.31	
2	26	60.7%	31.7%	0.05	
3	-	-	-	-	
4	-	-	-	-	
5	-	-	-	-	
Total	60	66.2%	38.5%	0.19	
Expert readers					
Modal Age	Otolith N	% Agreement	CV	Bias	
0	3	46.2%	110.7%	0.69	
1	42	60.5%	64.6%	0.02	
2	15	59.7%	34.0%	-0.23	
3	-	-	-	-	
4	-	-	-	-	
5	-	-	-	-	
Total	60	59.6%	59.2%	-0.01	

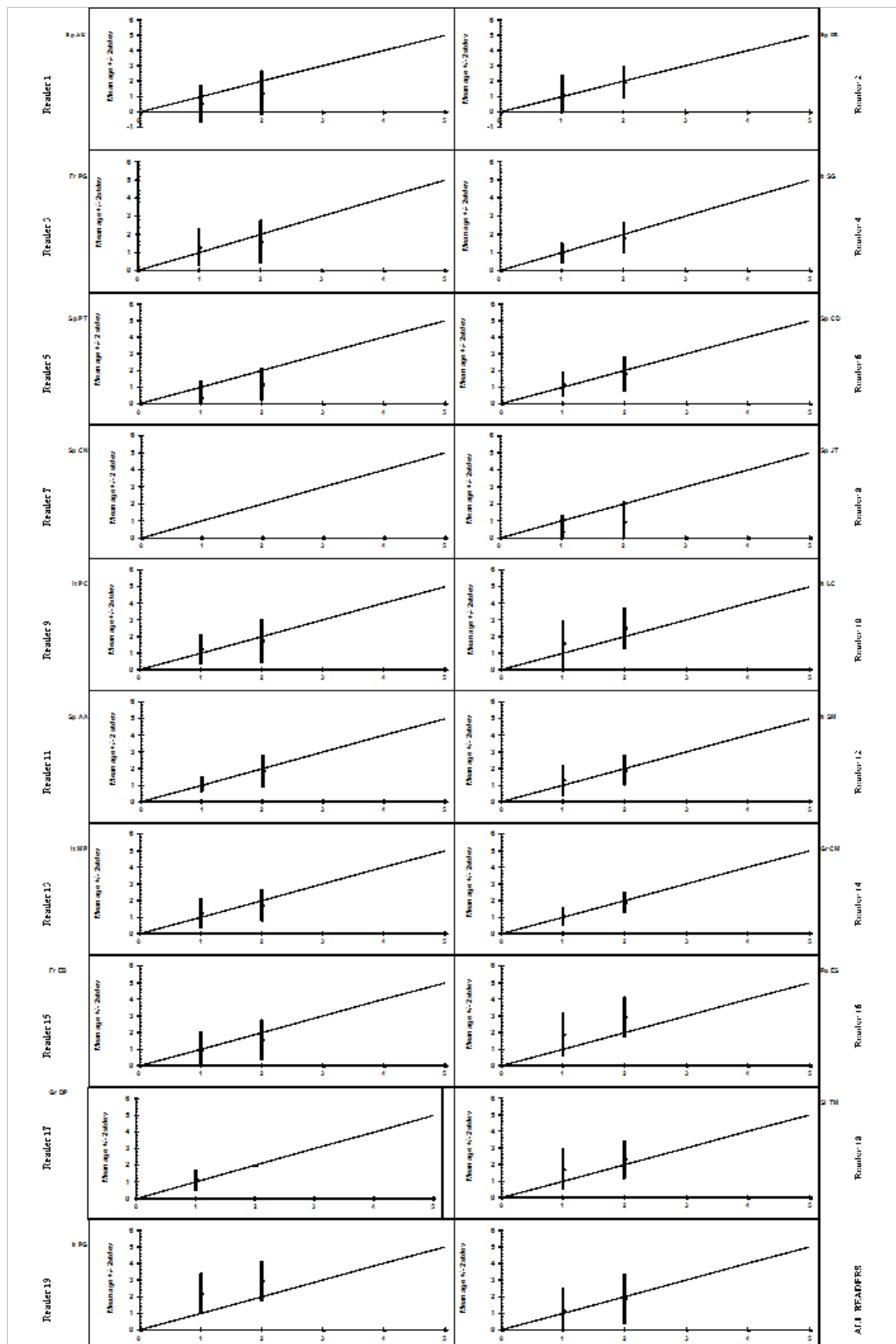


Figure: ANE_GSA06- Western Mediterranean. Age bias plots with the mean age recorded \pm 2stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

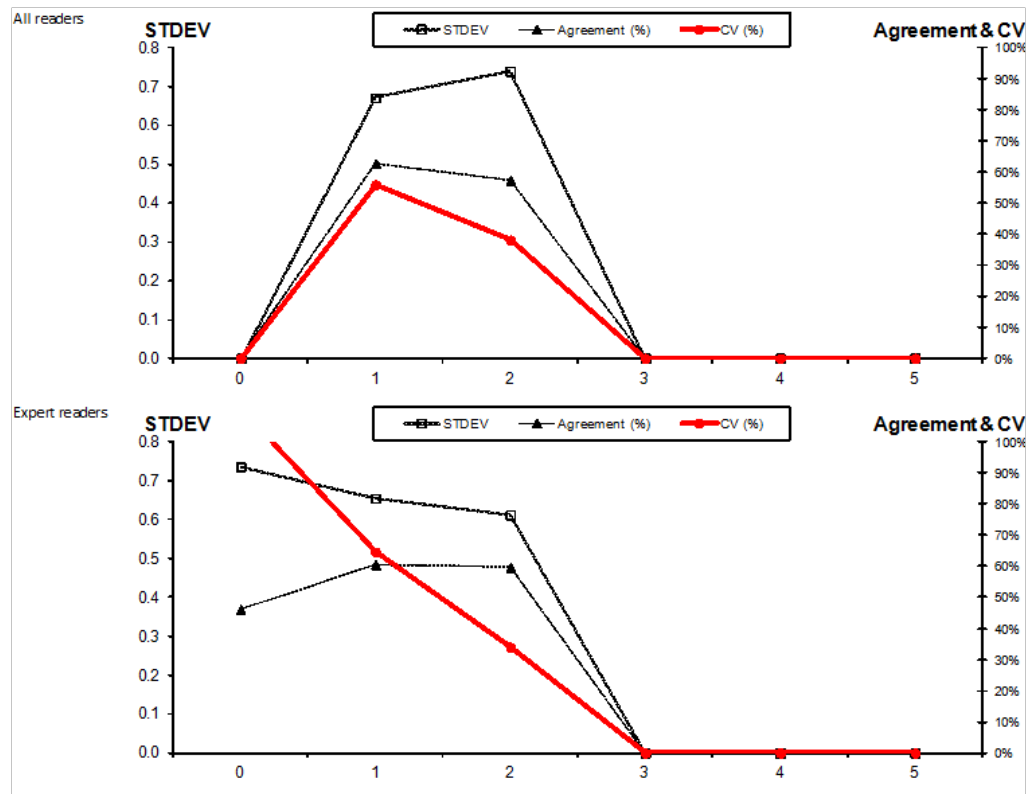


Figure: ANE_GSA06- Western Mediterranean. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.6 ANE_GSA07- Gulf of Lion

Table: Age reading results by reader

Table 1 Anchovy Otolith SET GSA07_Gulf of Lion (Anchovy Exchange 2014)																											RANGE r. 1-15					
Stratum		year	Sample no	Fish no	Fish length	Sex	Landing month	Sp AU Reader 1	Sp IR Reader 2	Fr PG Reader 3	It SG Reader 4	Sp PT Reader 5	Sp CD Reader 6	Sp CN Reader 7	Sp JT Reader 8	It PC Reader 9	It LC Reader 10	Sp AA Reader 11	It SM Reader 12	It MP Reader 13	Gr CM Reader 14	Fr EB Reader 15	Po ES Reader 16	Gr DP Reader 17	Si TM Reader 18	It PG Reader 19	MODAL age	Percent agreement	Precision CV			
Sem 1	2014	B	14_620_O_0020.jp	-	135.0	F	1	2	3	4	3	2	2	-	2	3	2	3	2	2	2	2	2	2	2	3	3	2	61%	25%		
Sem 1	2014	B	14_620_O_0042.jp	-	140.0	F	2	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	100%	0%		
Sem 1	2014	B	14_620_O_0043.jp	-	140.0	F	2	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	2	2	2	4	3	2	89%	24%		
Sem 1	2014	B	14_620_O_0046.jp	-	105.0	M	2	1	2	2	2	1	2	-	1	2	1	2	1	1	1	2	2	1	1	2	1	2	1	50%	34%	
Sem 1	2014	B	14_620_O_0049.jp	-	115.0	F	2	2	2	2	2	2	2	-	1	2	1	2	1	1	1	2	2	1	2	2	2	2	61%	31%		
Sem 1	2014	B	14_620_O_0050.jp	-	130.0	F	2	2	2	2	2	2	2	-	1	2	1	1	1	2	2	2	2	1	2	2	2	2	53%	38%		
Sem 1	2014	B	14_620_O_0055.jp	-	145.0	F	2	2	2	2	2	2	2	3	-	2	2	2	3	2	2	3	3	2	2	2	2	2	78%	19%		
Sem 1	2014	B	14_620_O_0059.jp	-	125.0	M	2	2	2	2	2	1	2	-	1	2	1	2	1	2	2	2	3	1	1	2	2	2	61%	33%		
Sem 1	2014	B	14_620_O_0090.jp	-	125.0	F	2	2	2	2	2	2	2	2	-	2	2	2	2	2	2	2	3	2	2	2	2	2	94%	11%		
Sem 1	2014	B	14_620_O_0094.jp	-	130.0	F	2	2	2	2	2	2	2	-	2	2	2	1	-	2	2	2	2	2	2	2	2	2	94%	12%		
Sem 1	2014	B	14_620_O_0095.jp	-	110.0	M	2	2	2	2	2	1	2	-	1	2	1	2	1	1	1	1	2	1	1	2	2	2	50%	34%		
Sem 1	2014	B	14_620_O_0099.jp	-	135.0	F	2	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	3	2	2	2	2	2	89%	17%		
Sem 1	2014	B	14_620_O_0076.jp	-	100.0	F	2	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100%	0%		
Sem 1	2014	B	14_620_O_0079.jp	-	120.0	F	2	2	2	2	2	2	2	-	2	2	1	2	1	2	1	2	2	2	2	3	2	2	83%	21%		
Sem 1	2014	B	14_620_O_0070.jp	-	105.0	undefined	2	2	2	2	2	1	2	-	1	2	1	2	1	1	1	1	2	2	1	1	1	1	2	50%	34%	
Sem 1	2014	B	14_620_O_0037.jp	-	110.0	M	2	2	2	2	1	1	2	-	1	1	1	2	1	1	1	1	1	1	1	2	1	2	1	67%	36%	
Sem 1	2014	B	14_620_O_0047.jp	-	115.0	F	2	2	2	2	2	1	2	-	1	2	1	2	1	1	1	1	2	2	1	1	2	2	56%	33%		
Sem 1	2014	B	14_620_O_0074.jp	-	120.0	F	2	3	2	3	2	1	2	-	2	1	1	2	1	1	1	1	1	2	1	2	2	2	44%	41%		
Sem 2	2014	B	14_618_O_0150.jp	-	94.0	M	7	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	0	1	2	1	89%	34%		
Sem 2	2014	B	14_618_O_0166.jp	-	100.0	M	7	1	1	1	0	1	1	-	1	1	1	1	1	1	1	1	1	1	1	0	2	1	83%	44%		
Sem 2	2014	B	14_618_O_0148.jp	-	111.0	undefined	7	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	2	1	94%	22%		
Sem 2	2014	B	14_618_O_0146.jp	-	111.0	M	7	1	1	1	1	1	1	-	1	1	1	2	1	1	1	1	1	1	1	1	2	1	89%	29%		
Sem 2	2014	B	14_618_O_0147.jp	-	112.0	M	7	1	1	1	1	1	1	-	1	1	1	2	1	1	1	1	1	1	1	1	2	1	89%	29%		
Sem 2	2014	B	14_618_O_0134.jp	-	118.0	M	7	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100%	0%		
Sem 2	2014	B	14_618_O_0142.jp	-	119.0	M	7	2	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	3	1	89%	44%		
Sem 2	2014	B	14_618_O_0139.jp	-	121.0	M	7	3	1	1	1	1	2	-	1	2	2	2	2	1	1	1	2	1	1	2	3	1	56%	45%		
Sem 2	2014	B	14_618_O_0133.jp	-	122.0	M	7	3	1	1	1	1	1	-	1	1	1	2	1	1	1	1	2	1	1	1	3	1	78%	51%		
Sem 2	2014	B	14_618_O_0135.jp	-	123.0	M	7	3	1	2	1	1	1	-	1	2	1	1	1	1	1	1	1	2	1	2	3	1	67%	49%		
Sem 2	2014	B	14_618_O_0136.jp	-	124.0	M	7	3	1	2	1	1	2	-	1	2	1	2	1	1	1	1	1	1	1	1	3	1	61%	47%		
Sem 2	2014	B	14_618_O_0127.jp	-	125.0	M	7	2	1	1	1	1	1	-	1	1	2	1	1	1	1	1	1	-	1	1	3	1	82%	46%		
Sem 2	2014	B	14_618_O_0130.jp	-	126.0	M	7	3	2	2	1	1	2	-	2	2	3	2	2	1	1	2	2	2	2	1	3	2	50%	36%		
Sem 2	2014	B	14_618_O_0129.jp	-	128.0	F	7	2	1	1	1	1	1	-	1	1	2	1	1	1	1	1	1	1	2	1	3	1	78%	46%		
Sem 2	2014	B	14_618_O_0124.jp	-	131.0	F	7	2	1	1	1	1	1	-	1	2	2	1	1	1	1	1	2	1	1	1	3	1	72%	46%		
Sem 2	2014	B	14_618_O_0177.jp	-	140.0	F	7	2	1	1	1	1	2	2	-	2	2	2	2	1	1	2	2	1	2	2	3	2	61%	33%		
Sem 2	2014	B	14_618_O_0178.jp	-	141.0	F	7	2	2	2	2	2	2	-	2	2	3	2	1	2	2	2	2	2	2	3	2	2	83%	20%		
Sem 2	2014	B	14_618_O_0175.jp	-	142.0	F	7	2	1	1	2	1	2	-	2	1	2	2	2	1	2	2	2	2	1	2	3	2	61%	33%		
Sem 2	2014	B	14_618_O_0176.jp	-	143.0	F	7	2	1	1	1	1	1	-	2	2	2	2	1	1	1	1	1	2	2	3	1	61%	43%			
Sem 2	2014	B	14_618_O_0179.jp	-	143.0	F	7	2	1	1	1	1	1	-	1	2	2	2	1	2	2	2	1	1	1	1	3	1	87%	44%		
Total read								38	38	38	38	38	38	0	38	38	38	37	38	38	38	37	38	38	38	37						
Total NOT read								0	0	0	0	0	0	38	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1		73.4%	31.3%

Table: ANE_GSA07- Gulf of Lion. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	-	-	-	-
1	19	77.4%	36.2%	0.25
2	19	69.4%	26.4%	-0.14
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	38	73.4%	31.3%	0.06
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	-	-	-	-
1	24	72.6%	39.4%	0.31
2	14	72.6%	25.0%	-0.03
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	38	72.6%	34.1%	0.19
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	-	-	-	-
1	17	75.8%	40.5%	0.32
2	21	74.5%	22.0%	-0.08
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	38	75.1%	30.3%	0.10

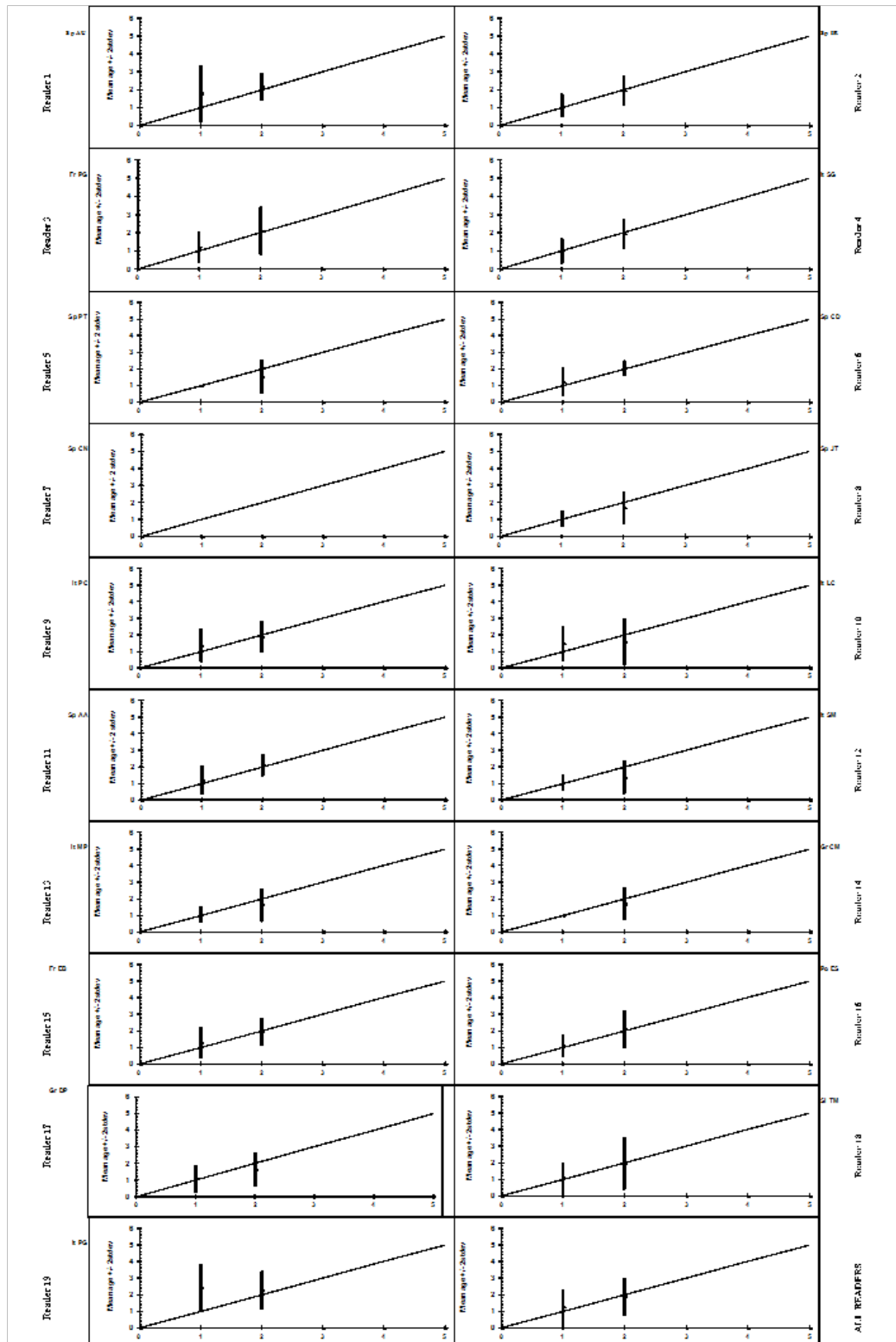


Figure: ANE_GSA07- Gulf of Lion. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

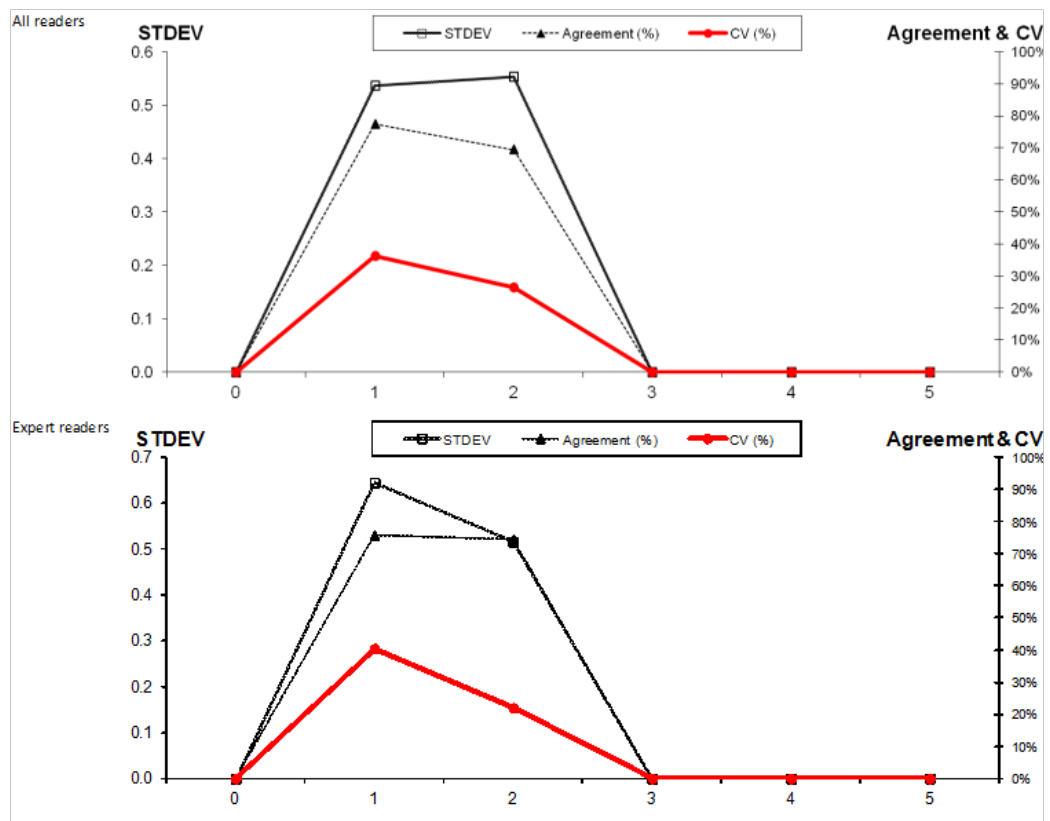


Figure: ANE_GSA07- Gulf of Lion. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.7 ANE_GSA10- Southern Tyrrhenian

Table: Age reading results by reader

Table 1		Anchovy Otolith SET GSA10_ Southern Thyrrhenian (Anchovy Exchange 2014)																				RANGE r. 1-15								
Stratum	year	Sample no	Fish no	Fish length	Sex	Landing month	Sp AU	Sp IR	Fr PG	It SG	Sp PT	Sp CD	Sp CN	Sp JT	It PC	It LC	Sp AA	It SM	It MP	Gr CM	Fr EB	Po ES	Gr DP	SITM	It PG	MODAL age	Percent agreement	Precision CV		
Sem 1	2012	GSA10_31.jpg	-	100.0	F	5	0	1	1	0	0	1	-	0	1	1	1	1	1	1	1	1	1	0	1	1	72%	64%		
Sem 1	2012	GSA10_32.jpg	-	100.0	M	5	0	1	1	0	0	1	-	0	0	1	1	1	1	1	1	1	1	-	1	1	71%	67%		
Sem 1	2012	GSA10_33.jpg	-	105.0	F	5	0	1	1	1	0	1	-	0	0	1	1	1	1	1	1	1	1	1	1	1	75%	55%		
Sem 1	2012	GSA10_34.jpg	-	105.0	M	5	0	1	2	0	0	1	-	0	0	1	1	1	1	1	1	1	1	1	1	1	67%	70%		
Sem 1	2012	GSA10_35.jpg	-	110.0	M	5	0	1	1	1	0	1	-	0	0	1	1	1	1	1	1	1	1	1	2	1	72%	62%		
Sem 1	2012	GSA10_36.jpg	-	110.0	F	5	0	1	1	0	0	1	-	0	0	1	1	1	1	1	1	1	1	1	0	1	67%	73%		
Sem 1	2012	GSA10_37.jpg	-	115.0	F	5	0	1	1	1	0	1	-	0	0	1	1	1	1	1	1	1	1	1	2	1	72%	62%		
Sem 1	2012	GSA10_38.jpg	-	115.0	F	5	0	1	1	1	0	0	-	0	0	1	0	1	1	1	1	1	1	2	2	1	55%	83%		
Sem 1	2012	GSA10_39.jpg	-	115.0	M	5	0	1	1	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	2	1	75%	53%		
Sem 1	2012	GSA10_40.jpg	-	115.0	M	5	0	1	1	1	0	0	-	0	0	0	0	1	1	1	1	1	0	1	2	1	50%	96%		
Sem 1	2012	GSA10_41.jpg	-	120.0	M	5	0	1	1	1	0	1	-	0	0	1	1	1	1	1	1	1	1	1	2	1	72%	62%		
Sem 1	2012	GSA10_42.jpg	-	120.0	M	5	0	1	1	1	0	1	-	0	0	1	0	1	1	1	1	1	1	1	1	1	72%	64%		
Sem 1	2012	GSA10_43.jpg	-	120.0	F	5	0	1	1	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	1	1	83%	46%		
Sem 1	2012	GSA10_44.jpg	-	120.0	F	5	0	1	2	1	0	1	-	0	1	1	1	1	1	1	1	1	1	2	1	1	72%	57%		
Sem 1	2012	GSA10_45.jpg	-	125.0	F	5	0	1	2	1	0	0	-	0	0	1	0	1	1	1	1	1	0	1	0	2	0	44%	103%	
Sem 1	2012	GSA10_46.jpg	-	125.0	F	5	0	1	1	2	0	1	-	0	1	1	1	2	2	2	2	1	1	2	2	2	1	44%	60%	
Sem 1	2012	GSA10_47.jpg	-	125.0	M	5	0	1	1	1	0	1	-	0	1	2	1	2	2	1	1	1	1	1	1	2	1	67%	52%	
Sem 1	2012	GSA10_48.jpg	-	125.0	M	5	0	1	1	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	1	2	1	75%	53%	
Sem 1	2012	GSA10_49.jpg	-	130.0	F	5	0	1	1	1	0	0	-	0	1	1	0	1	1	1	1	1	1	1	2	1	67%	70%		
Sem 1	2012	GSA10_50.jpg	-	130.0	F	5	0	1	1	1	0	0	-	0	1	1	0	1	1	1	1	1	1	1	2	2	1	61%	74%	
Sem 1	2012	GSA10_51.jpg	-	130.0	F	5	0	1	1	2	0	1	-	0	1	2	1	2	2	2	2	1	1	1	2	2	2	44%	58%	
Sem 1	2012	GSA10_52.jpg	-	95.0	F	5	0	1	1	0	0	0	-	0	0	0	0	0	0	0	0	1	1	0	1	0	61%	129%		
Sem 1	2012	GSA10_53.jpg	-	95.0	F	5	0	1	1	0	0	0	-	0	1	1	0	0	0	0	1	1	1	0	0	1	0	55%	115%	
Sem 1	2012	GSA10_54.jpg	-	95.0	M	5	0	1	1	0	0	0	-	0	0	0	0	0	0	0	1	1	1	0	0	1	0	67%	145%	
Sem 1	2012	GSA10_55.jpg	-	95.0	M	5	0	0	1	0	0	0	-	0	0	0	0	0	0	0	0	1	1	0	0	1	0	78%	130%	
Sem 2	2013	GSA10_01.jpg	-	110.0	F	8	1	2	2	2	1	2	-	1	1	3	2	1	1	2	1	3	1	2	3	1	44%	44%		
Sem 2	2013	GSA10_02.jpg	-	110.0	F	8	1	2	1	1	1	0	-	1	1	2	2	1	1	1	1	1	2	1	2	2	1	61%	45%	
Sem 2	2013	GSA10_03.jpg	-	110.0	M	8	1	2	1	1	1	2	-	1	1	2	2	1	1	1	1	1	2	1	2	2	1	61%	36%	
Sem 2	2013	GSA10_04.jpg	-	115.0	M	8	1	2	1	2	1	2	-	1	1	2	2	1	1	1	1	1	2	1	2	2	1	55%	35%	
Sem 2	2013	GSA10_05.jpg	-	115.0	M	8	-	2	1	2	1	2	-	1	1	2	2	1	1	1	1	1	2	1	2	2	1	53%	35%	
Sem 2	2013	GSA10_06.jpg	-	115.0	F	8	1	2	1	2	1	2	-	1	1	3	2	1	1	1	1	1	2	1	2	2	1	55%	41%	
Sem 2	2013	GSA10_07.jpg	-	130.0	M	8	2	3	2	2	2	2	-	2	2	3	2	2	2	2	2	2	3	2	3	3	2	72%	20%	
Sem 2	2013	GSA10_08.jpg	-	135.0	M	8	1	2	1	2	1	2	-	1	1	2	2	2	2	2	2	1	2	1	2	2	2	61%	31%	
Sem 2	2013	GSA10_09.jpg	-	135.0	M	8	2	2	2	2	2	2	-	2	2	2	2	2	2	2	2	1	3	2	2	2	2	89%	17%	
Sem 2	2013	GSA10_10.jpg	-	140.0	M	8	2	2	2	2	2	2	-	2	3	3	2	2	2	2	2	2	1	3	3	3	2	67%	25%	
Sem 2	2013	GSA10_11.jpg	-	145.0	M	8	1	3	2	2	2	2	-	1	2	3	2	2	2	2	2	1	3	2	2	3	2	61%	31%	
Sem 2	2013	GSA10_12.jpg	-	150.0	F	8	2	3	2	2	2	2	-	2	2	3	2	2	2	2	2	2	3	2	3	3	2	72%	20%	
Sem 2	2012	GSA10_13.jpg	-	125.0	M	9	1	2	2	1	1	2	-	1	1	2	2	1	1	1	1	1	3	1	2	2	1	55%	41%	
Sem 2	2012	GSA10_14.jpg	-	125.0	F	9	1	2	3	2	1	2	-	1	2	3	2	1	1	1	2	1	4	1	3	3	1	39%	48%	
Sem 2	2012	GSA10_15.jpg	-	135.0	M	9	1	2	2	2	1	2	-	1	2	3	2	1	1	2	1	3	2	3	3	3	2	44%	40%	
Sem 2	2012	GSA10_16.jpg	-	140.0	F	9	1	2	1	2	1	2	-	1	2	2	2	1	1	2	1	3	2	2	3	3	2	50%	38%	
Sem 2	2012	GSA10_17.jpg	-	140.0	M	9	1	2	1	2	2	2	-	1	2	2	2	2	2	2	2	1	3	2	3	3	2	61%	33%	
Sem 2	2012	GSA10_18.jpg	-	145.0	M	9	1	2	2	2	2	2	-	1	2	2	2	2	2	2	2	2	1	3	2	3	3	2	67%	30%
Sem 2	2012	GSA10_19.jpg	-	145.0	M	9	2	1	2	2	2	2	-	1	2	2	2	2	2	2	2	1	3	2	3	3	2	61%	33%	
Sem 2	2012	GSA10_20.jpg	-	145.0	F	9	1	2	1	2	2	2	-	2	2	2	2	2	2	2	2	2	1	2	2	3	2	72%	28%	
Sem 2	2012	GSA10_21.jpg	-	150.0	F	9	1	2	1	2	2	2	-	1	2	2	2	2	2	2	2	2	1	2	2	3	2	67%	31%	
Sem 2	2012	GSA10_22.jpg	-	150.0	F	9	1	2	1	2	2	2	-	2	2	2	2	2	2	2	2	2	1	2	1	2	3	2	72%	28%
Sem 2	2012	GSA10_23.jpg	-	80.0	U	8	0	1	0	0	0	1	-	1	0	0	0	1	0	0	0	0	1	0	1	0	0	67%	148%	
Sem 2	2012	GSA10_24.jpg	-	80.0	U	8	0	1	0	0	0	0	1	-	1	0	1	1	0	0	0	0	1	0	0	1	0	61%	129%	
Sem 2	2012	GSA10_25.jpg	-	85.0	U	8	0	1	0	0	0	1	-	1	0	1	1	0	0	0	0	0	1	0	0	1	0	61%	129%	
Sem 2	2012	GSA10_26.jpg	-	85.0	U	8	0	1	0	0	0	0	1	-	1	0	1	1	0	0	0	0	1	0	0	0	0	67%	148%	
Sem 2	2012	GSA10_27.jpg	-	90.0	F	8	0	1	0	0	0	0	1	-	1	0	1	1	0	0	0	0	1	0	0	1	0	61%	129%	
Sem 2	2012	GSA10_28.jpg	-	90.0	F	8	0	1	0	0	0	0	1	-	1	0	1	1	1	0	0	0	1	0	1	1	0	50%	103%	
Sem 2	2012	GSA10_29.jpg	-	90.0	M	8	0	1	0	0	0	0	1	-	1	0	1	1	1	0	0	0	1	0	1	0	0	50%	103%	
Sem 2	2012	GSA10_30.jpg	-	90.0	M	8	0	1	0	0	-	0	1	-	1	0	1	1	1	0	0	0	1	0	1	0	0	53%	108%	
Total read							54	55	55	54	55	55	0	55	55	55	55	55	55	55	55	55	55	55	54	55	0			
Total NOT read							1	0	0	1	0	0	55	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	62.9%	67.2%

Table: ANE_GSA10- Southern Tyrrhenian. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	13	59.7%	129.1%	0.41
1	27	63.8%	57.5%	0.04
2	15	64.1%	31.0%	-0.07
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	55	62.9%	67.2%	0.10
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	8	61.1%	136.4%	0.39
1	31	73.8%	40.4%	0.11
2	15	65.2%	28.6%	0.02
3	1	33.3%	-	-1.00
4	-	-	-	-
5	-	-	-	-
Total	55	68.9%	51.2%	0.11
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	20	62.9%	147.4%	0.39
1	19	57.9%	71.2%	-0.11
2	16	65.7%	29.2%	-0.15
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	55	62.0%	86.7%	0.06
Tyrrhenian readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	72.2%	144.3%	0.33
1	25	65.3%	66.8%	-0.08
2	17	66.7%	29.4%	-0.02
3	7	71.4%	22.5%	-0.33
4	-	-	-	-
5	-	-	-	-
Total	55	67.3%	58.1%	-0.05

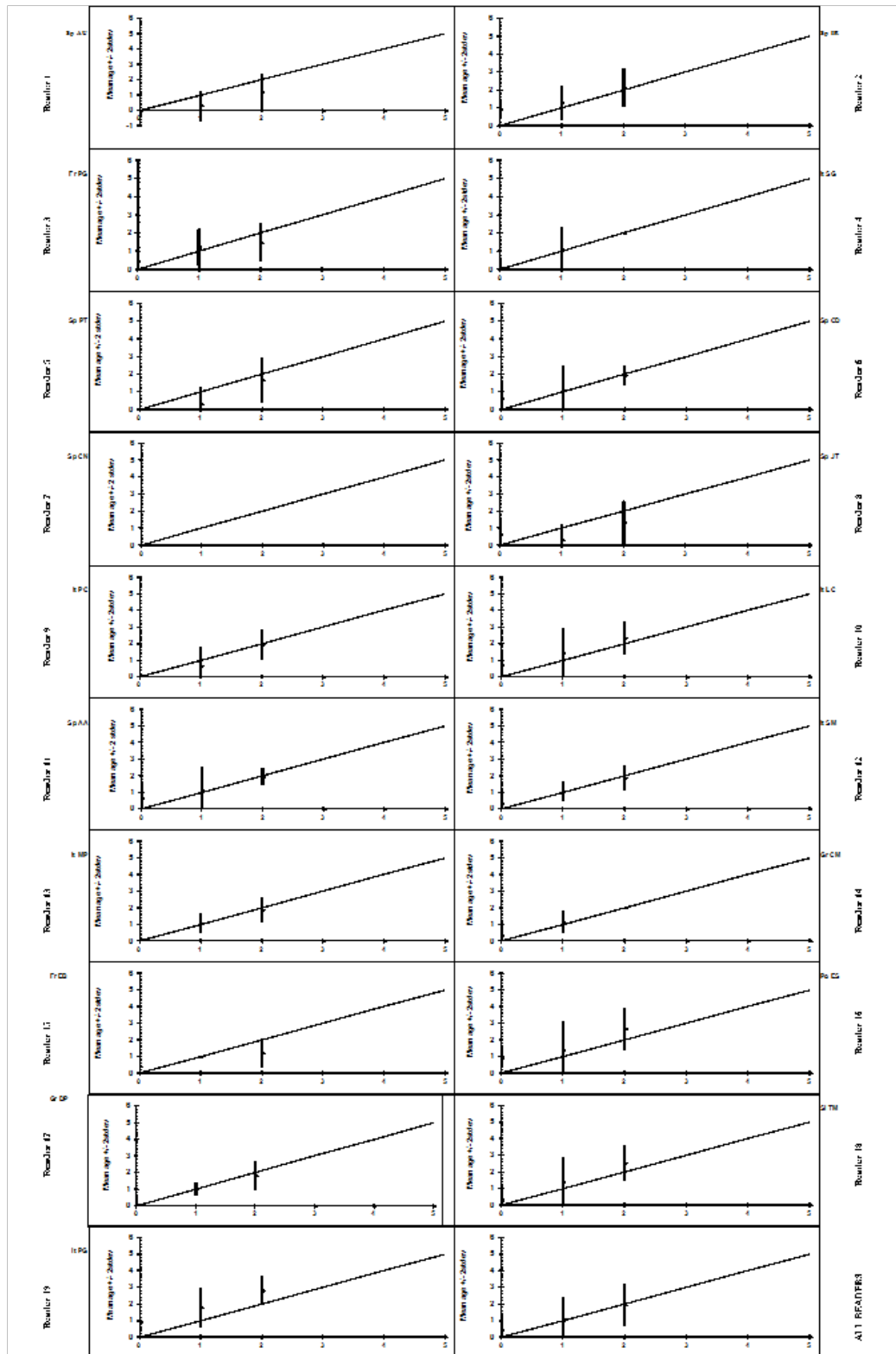


Figure: ANE_GSA10- Southern Tyrrhenian. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

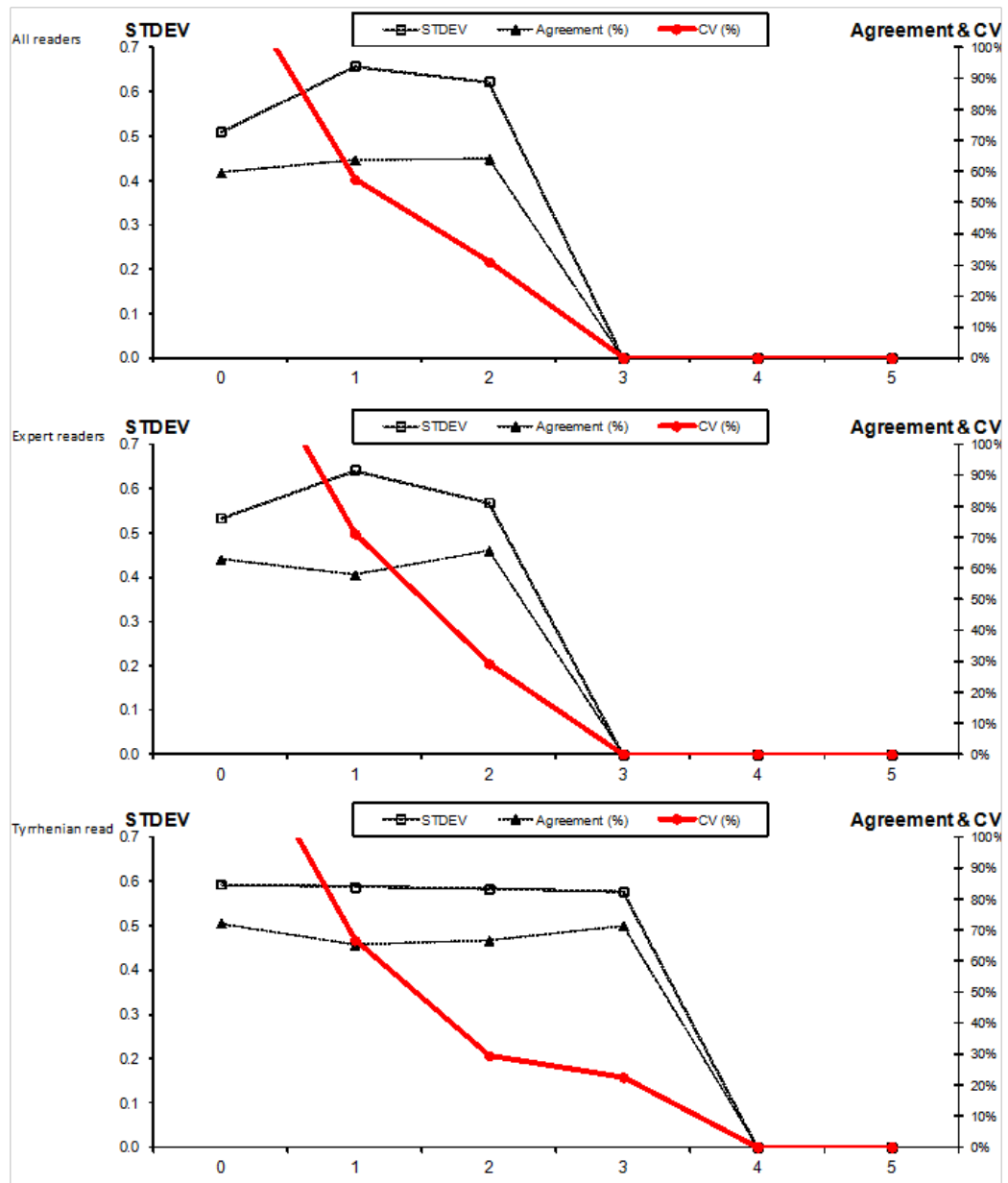


Figure: ANE_GSA10- Southern Tyrrhenian. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.8 ANE_GSA16- Strait of Sicily

Table: Age reading results by reader

Table 1		Anchovy Otolith SET GSA16-Strait of Sicily (Anchovy Exchange 2014)																								RANGE r. 1-15			
Stratum	year	Sample no	Flan no	Flan length	Sex	Landing month	Sp AU	Sp IR	Fr PG	It SG	Sp PT	Sp CD	Sp CN	Sp JT	It PC	It LC	Sp AA	It SM	It MP	Gr CM	Fr EB	Fr ES	Gr DP	It TM	It PG	MODAL age	Percent age	Precision CV	
Sem 1	2010	CB201010-5-10_6(40).jpg	-	96.0	undefined	5	-	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94%	412%
Sem 1	2010	CB201010-5-10_15(40).jpg	-	96.0	undefined	5	0	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94%	424%
Sem 1	2010	CB201010-5-10_27(40).jpg	-	86.0	undefined	5	0	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94%	424%
Sem 1	2010	CB201010-5-10_28(40).jpg	-	94.0	undefined	5	0	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	89%	291%
Sem 1	2010	CB201010-5-10_40(40).jpg	-	90.0	undefined	5	0	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94%	424%
Sem 1	2010	CB201010-5-10_47(40).jpg	-	83.0	undefined	5	-	0	1	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94%	412%
Sem 1	2010	CB201010-5-10_53(2).jpg	-	131.0	male	4	1	1	2	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	2	2	1	72%	51%
Sem 1	2010	CB201010-5-10_27(40).jpg	-	116.0	male	4	1	1	2	1	0	1	-	1	1	1	1	1	1	2	1	1	1	1	1	1	1	72%	44%
Sem 1	2010	CB201010-5-10_16(40).jpg	-	114.0	male	5	0	0	2	1	0	1	-	0	2	1	1	1	1	1	1	1	1	1	1	1	1	67%	66%
Sem 1	2010	CB201010-5-10_17(40).jpg	-	106.0	male	5	0	0	1	1	0	0	-	0	0	0	0	0	1	0	1	0	0	0	0	1	0	67%	146%
Sem 1	2010	CB201010-5-10_19(40).jpg	-	112.0	male	5	0	0	1	1	0	1	-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	78%	56%
Sem 1	2010	CB201010-5-10_36(40).jpg	-	106.0	female	5	1	1	2	1	0	1	-	0	2	1	1	1	1	1	1	1	1	1	2	2	1	67%	52%
Sem 1	2010	CB201010-5-10_21(32).jpg	-	131.0	male	4	1	1	2	1	1	0	-	1	1	2	1	1	1	1	1	2	1	2	3	1	1	67%	52%
Sem 1	2010	CB201010-5-10_11(32).jpg	-	132.0	female	4	0	1	2	1	1	1	-	1	2	2	1	1	1	1	2	2	1	2	3	1	1	56%	50%
Sem 1	2010	CB201010-5-10_16(32).jpg	-	133.0	male	4	0	1	2	1	1	1	-	1	2	2	1	1	1	2	2	2	1	1	3	1	1	56%	50%
Sem 1	2010	CB201010-5-10_16(32).jpg	-	129.0	male	4	0	1	1	1	1	1	-	1	1	1	1	1	1	1	2	2	2	1	2	3	1	72%	53%
Sem 1	2010	CB201010-5-10_19(32).jpg	-	149.0	female	4	1	1	2	2	1	1	-	1	2	2	1	1	2	1	2	2	2	1	2	3	1	50%	40%
Sem 1	2010	CB201010-5-10_26(32).jpg	-	141.0	male	4	0	1	1	1	1	1	-	1	1	2	1	1	1	1	2	3	1	2	3	1	1	67%	56%
Sem 1	2010	CB201010-5-10_27(32).jpg	-	126.0	male	4	0	1	2	1	0	1	-	0	2	1	1	1	1	1	2	2	1	2	2	1	1	56%	61%
Sem 1	2010	CB201010-5-10_11(32).jpg	-	126.0	male	5	0	1	1	1	1	1	-	1	1	1	1	1	1	1	2	2	1	2	2	1	1	72%	44%
Sem 1	2010	CB201010-5-10_9(32).jpg	-	130.0	male	5	2	1	3	1	1	1	-	1	2	2	1	1	1	2	2	3	1	2	2	1	1	60%	43%
Sem 1	2010	CB201010-5-10_14(32).jpg	-	128.0	male	5	0	1	2	1	1	1	-	1	1	1	1	1	1	1	2	1	1	1	2	2	1	72%	44%
Sem 1	2010	CB201010-5-10_24(32).jpg	-	136.0	female	5	0	1	3	1	1	1	-	1	2	2	1	1	1	2	2	2	1	2	2	1	1	50%	49%
Sem 1	2010	CB201010-5-10_31(32).jpg	-	147.0	female	4	2	1	2	2	1	2	-	1	3	2	2	2	2	2	2	2	2	2	3	2	2	72%	26%
Sem 1	2010	CB201010-5-10_17(32).jpg	-	144.0	female	4	2	1	1	2	2	1	-	1	2	2	1	1	1	1	2	2	1	2	3	1	1	44%	50%
Sem 1	2010	CB201010-5-10_22(32).jpg	-	144.0	female	4	1	1	2	2	1	1	-	1	3	3	1	2	2	2	2	2	4	1	3	3	1	39%	46%
Sem 1	2010	CB201010-5-10_23(32).jpg	-	143.0	male	4	0	1	3	2	2	1	-	1	2	2	1	2	2	2	1	3	1	2	3	1	1	39%	50%
Sem 1	2010	CB201010-5-10_31(32).jpg	-	158.0	male	4	1	1	2	2	1	1	-	1	2	3	1	2	2	2	2	2	2	1	3	4	2	44%	47%
Sem 1	2010	CB201010-5-10_31(32).jpg	-	135.0	female	4	1	1	1	1	1	1	-	0	3	1	1	2	2	2	2	2	2	1	3	2	1	50%	52%
Sem 1	2010	CB201010-5-10_16(32).jpg	-	133.0	female	5	1	1	3	1	1	1	-	1	3	2	1	2	2	3	2	3	1	2	3	1	1	44%	47%
Sem 1	2010	CB201010-5-10_23(32).jpg	-	140.0	female	5	2	1	2	2	1	1	-	0	2	2	2	2	2	2	2	3	2	2	2	2	2	72%	36%
Sem 1	2010	CB201010-5-10_14(32).jpg	-	163.0	female	4	2	2	3	2	2	1	-	1	3	4	2	2	3	3	3	3	2	1	3	4	2	39%	36%
Sem 2	2010	CB201010-5-10_27(40).jpg	-	119.0	undefined	9	0	1	1	1	0	2	-	0	1	2	2	1	1	1	1	1	3	1	2	3	1	50%	70%
Sem 2	2010	CB201010-5-10_21(32).jpg	-	126.0	undefined	9	1	3	2	1	1	2	-	1	-	2	2	1	1	2	1	3	1	2	3	1	1	47%	46%
Sem 2	2010	CB201010-5-10_14(32).jpg	-	144.0	female	9	1	2	4	2	1	2	-	1	2	3	2	1	1	2	1	4	1	3	4	1	1	39%	68%
Sem 2	2010	CB201010-5-10_34(32).jpg	-	146.0	undefined	9	1	2	2	2	2	2	-	1	3	3	2	2	2	2	2	1	3	2	3	4	2	66%	36%
Sem 2	2010	CB201010-5-10_7(32).jpg	-	138.0	female	9	2	2	1	1	1	2	-	1	2	2	2	2	2	2	2	1	3	2	2	3	2	61%	34%
Sem 2	2010	CB201010-5-10_10(32).jpg	-	145.0	undefined	9	1	-	2	2	2	2	-	1	2	3	2	2	2	2	2	2	3	2	3	4	2	66%	33%
Sem 2	2010	CB201010-5-10_26(32).jpg	-	141.0	female	9	2	2	2	2	1	2	-	1	2	3	3	2	2	2	2	2	4	2	2	4	2	67%	36%
Sem 2	2010	CB201010-5-10_31(32).jpg	-	161.0	female	9	2	3	1	3	2	2	-	1	2	4	3	2	2	2	2	1	3	2	2	4	2	50%	39%
Sem 2	2010	CB201010-5-10_21(32).jpg	-	142.0	male	9	1	2	2	2	3	2	-	2	2	3	2	1	2	3	1	3	2	2	3	2	2	66%	32%
Sem 2	2010	CB201010-5-10_6(32).jpg	-	126.0	undefined	9	0	1	1	2	1	2	-	1	2	2	2	1	1	1	1	3	1	2	2	1	1	50%	49%
Sem 2	2010	CB201010-5-10_13(32).jpg	-	116.0	undefined	9	0	1	1	1	1	2	-	1	1	2	2	1	1	1	1	2	1	2	2	1	1	61%	45%
Sem 2	2010	CB201010-5-10_15(32).jpg	-	126.0	undefined	9	0	1	1	1	1	2	-	1	1	2	2	1	1	1	1	1	3	1	2	3	1	61%	56%
Sem 2	2010	CB201010-5-10_20(32).jpg	-	116.0	undefined	9	1	1	1	2	1	2	-	1	1	1	2	1	1	1	1	1	2	1	2	2	1	67%	36%
Sem 2	2010	CB201010-5-10_27(32).jpg	-	131.0	undefined	9	-	1	1	2	1	2	-	1	1	2	2	1	1	1	1	1	2	1	2	2	1	59%	36%
Sem 2	2010	CB201010-5-10_11(32).jpg	-	116.0	undefined	9	0	1	0	1	0	1	-	0	1	1	1	1	1	1	-	1	0	1	2	1	1	66%	74%
Sem 2	2010	CB201010-5-10_5(32).jpg	-	130.0	undefined	9	1	2	2	2	1	2	-	1	2	3	2	1	1	2	1	2	2	2	3	2	2	66%	36%
Sem 2	2010	CB201010-5-10_20(32).jpg	-	126.0	undefined	9	1	1	1	1	1	2	-	1	1	2	2	1	1	1	1	3	1	3	3	1	1	67%	52%
Sem 2	2010	CB201010-5-10_11(32).jpg	-	133.0	undefined	9	2	2	1	1	1	2	-	1	2	2	2	1	1	2	1	2	1	2	3	2	2	44%	41%
Sem 2	2010	CB201010-5-10_13(32).jpg	-	121.0	undefined	9	2	2	1	1	1	2	-	1	1	2	2	1	1	1	1	3	1	2	3	1	1	56%	45%
Sem 2	2																												

Table: ANE_GSA16- Strait of Sicily. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	7	89.5%	362.0%	0.10
1	35	57.9%	50.8%	0.38
2	21	50.5%	37.5%	0.17
3	2	48.6%	29.9%	-0.49
4	-	-	-	-
5	-	-	-	-
Total	65	58.5%	78.7%	0.26
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	7	92.1%	59.8%	0.08
1	27	64.5%	41.9%	0.47
2	28	52.6%	35.6%	0.23
3	4	41.7%	34.6%	-0.56
4	-	-	-	-
5	-	-	-	-
Total	66	61.0%	40.7%	0.26
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	9	75.9%	239.8%	0.30
1	37	58.3%	55.1%	0.31
2	17	55.9%	34.2%	-0.07
3	3	53.8%	31.5%	-0.50
4	-	-	-	-
5	-	-	-	-
Total	66	59.9%	73.8%	0.18
Sicilian readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	100.0%	0.0%	0.00
1	27	100.0%	0.0%	0.00
2	26	76.9%	20.9%	-0.19
3	6	50.0%	28.3%	-0.50
4	-	-	-	-
5	-	-	-	-
Total	66	85.6%	11.2%	-0.13

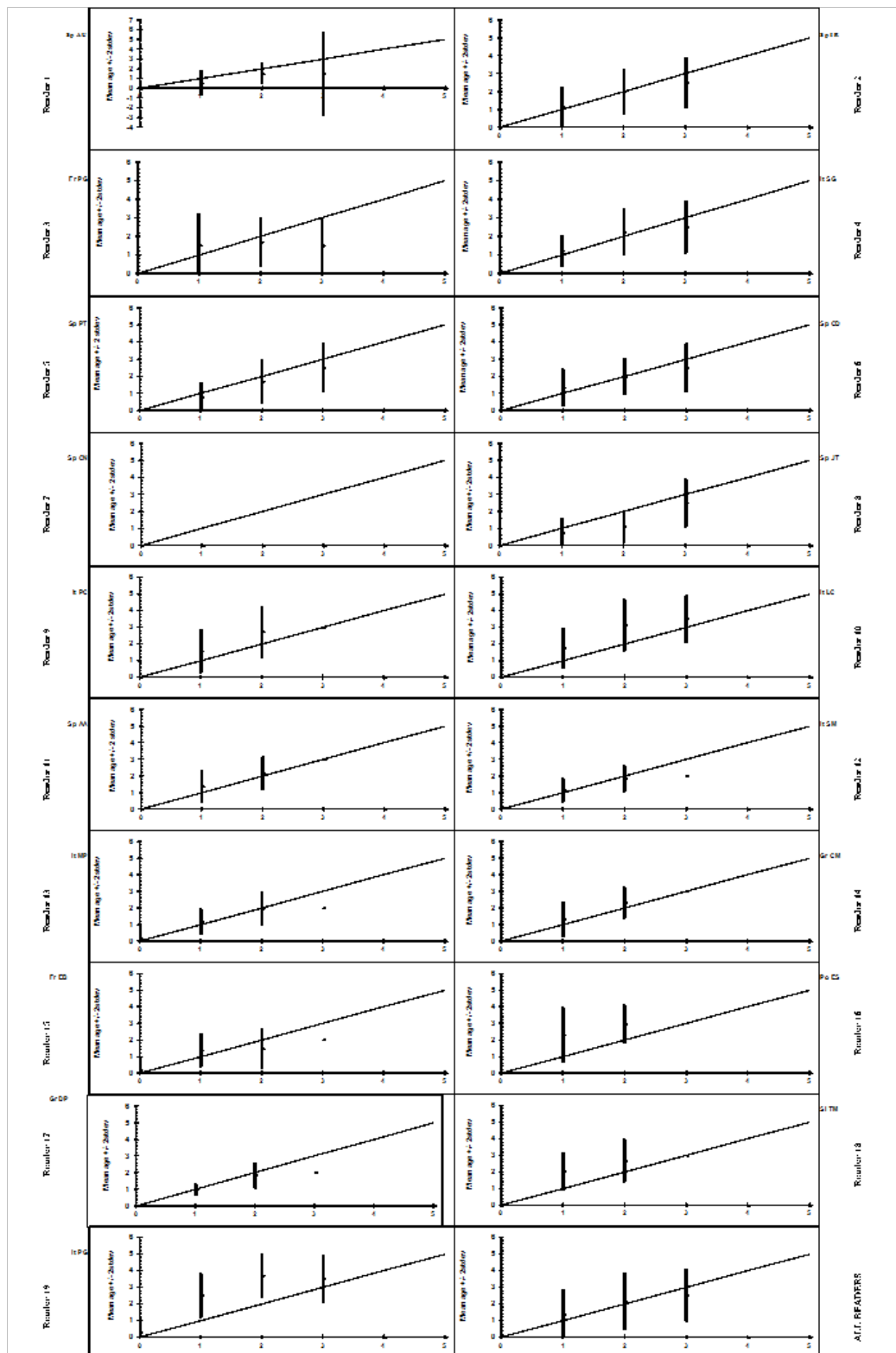


Figure: ANE_GSA16- Strait of Sicily. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

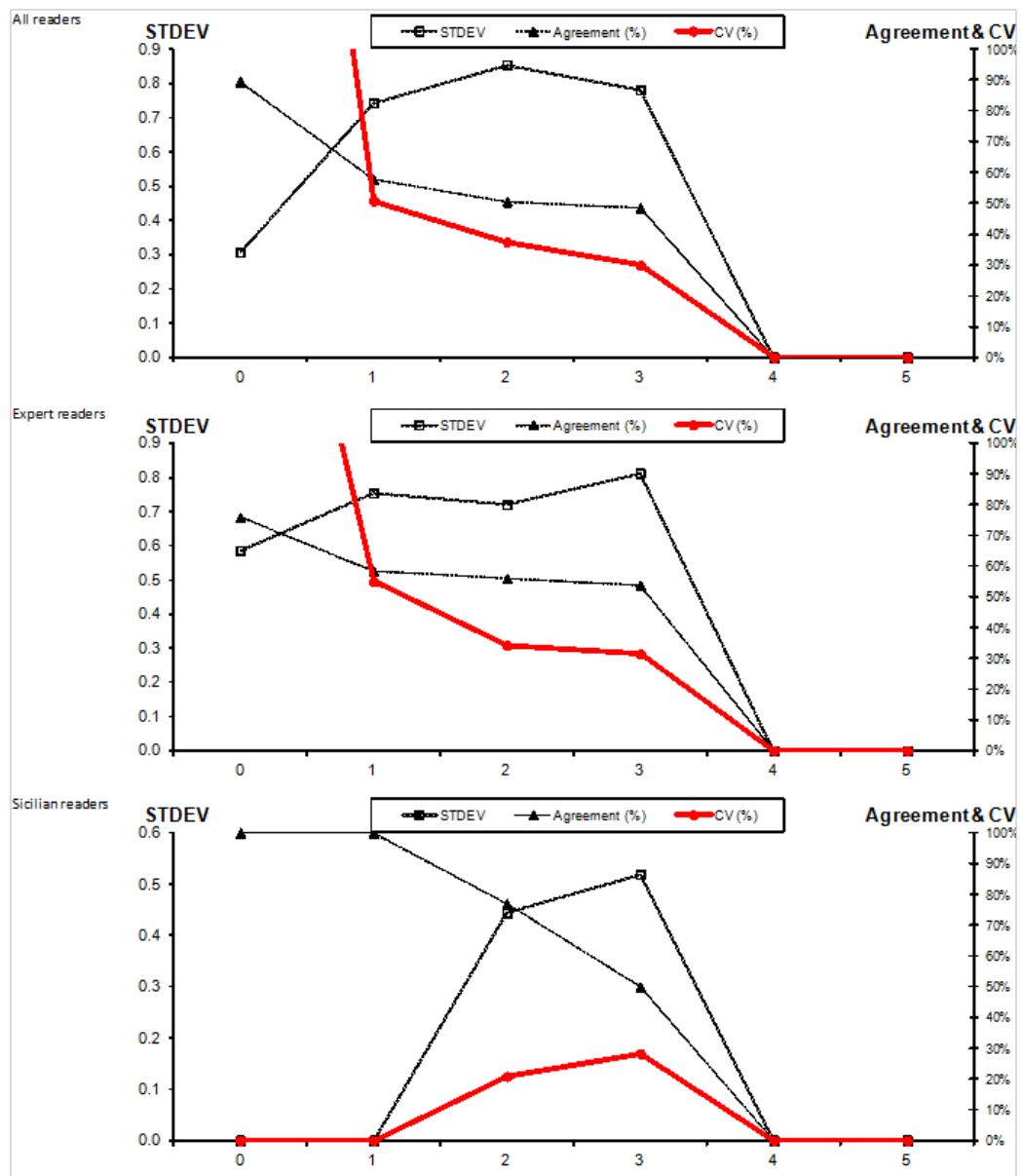


Figure: ANE_GSA16- Strait of Sicily. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.9 ANE_GSA19- Western Ionian

Table: Age reading results by reader

Table 1		Anchovy Otolith SET GSA19-Western Ionian (Anchovy Exchange 2014)																								RANGE r. 1-15					
Station	year	Sample no	Fish no	Fish length	Sex	Landing month	Sp AU	Sp IR	Fr PG	t SG	Sp PT	Sp CD	Sp CN	Sp JT	t PC	t LC	Sp AA	t SM	t MP	Gr CM	Fr EB	Sp ES	Gr DP	Si TM	t PG	MODAL	Percent	Precision			
							Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	age	agreement	CV			
Sem 1	2012	GSA19_33.jpg	-	125.0	M	5	2	1	2	1	0	0	-	1	0	1	0	1	1	1	1	1	1	1	1	1	1	67%	66%		
Sem 1	2012	GSA19_34.jpg	-	125.0	F	5	0	1	2	1	0	1	-	0	1	1	1	1	1	1	2	1	1	1	1	1	1	1	72%	57%	
Sem 1	2012	GSA19_35.jpg	-	130.0	F	5	2	1	2	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	83%	33%	
Sem 1	2012	GSA19_36.jpg	-	130.0	M	5	2	-	2	1	1	1	-	1	1	2	1	1	1	1	2	3	2	2	2	2	2	2	47%	39%	
Sem 1	2012	GSA19_37.jpg	-	135.0	F	5	2	2	2	2	1	1	-	1	1	2	1	1	1	1	2	2	2	1	1	2	2	2	50%	34%	
Sem 1	2012	GSA19_38.jpg	-	135.0	M	5	2	2	2	1	0	1	-	0	1	1	1	0	1	2	1	2	1	1	1	1	1	1	56%	61%	
Sem 1	2012	GSA19_39.jpg	-	140.0	F	5	1	1	2	2	1	1	-	1	1	1	1	1	2	1	2	2	1	1	2	2	1	1	67%	36%	
Sem 1	2012	GSA19_40.jpg	-	140.0	M	5	1	1	2	2	1	1	-	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	72%	35%	
Sem 1	2012	GSA19_41.jpg	-	145.0	F	5	1	1	2	2	1	1	-	0	2	2	2	1	2	2	2	2	2	2	2	2	2	2	67%	38%	
Sem 1	2012	GSA19_42.jpg	-	100.0	M	4	0	1	2	1	0	1	-	0	0	0	1	1	1	1	1	1	2	1	1	1	1	1	61%	74%	
Sem 1	2012	GSA19_43.jpg	-	105.0	F	4	0	1	2	1	0	1	-	0	0	0	1	1	1	1	1	1	2	1	1	1	1	1	61%	74%	
Sem 1	2012	GSA19_44.jpg	-	105.0	M	4	0	1	1	1	0	0	-	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	56%	92%	
Sem 1	2012	GSA19_45.jpg	-	110.0	M	4	0	1	1	0	0	1	-	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	67%	73%	
Sem 1	2012	GSA19_46.jpg	-	110.0	F	4	0	1	2	0	0	1	-	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	56%	89%	
Sem 1	2012	GSA19_47.jpg	-	115.0	F	4	0	1	1	0	0	1	-	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	67%	73%	
Sem 1	2012	GSA19_48.jpg	-	115.0	M	4	0	1	2	0	0	1	-	0	1	1	1	1	1	1	1	1	0	1	0	1	1	1	61%	80%	
Sem 1	2012	GSA19_49.jpg	-	120.0	F	4	1	1	3	1	1	1	-	0	2	1	1	1	1	2	2	2	1	2	2	2	1	1	50%	49%	
Sem 1	2012	GSA19_50.jpg	-	120.0	M	4	1	1	2	1	0	1	-	1	1	1	1	1	1	2	1	2	2	1	2	1	1	1	67%	45%	
Sem 1	2012	GSA19_51.jpg	-	100.0	F	4	-	1	1	0	0	1	-	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	65%	76%	
Sem 1	2012	GSA19_52.jpg	-	135.0	F	5	0	1	1	1	1	1	-	0	1	1	1	1	1	1	1	1	2	1	1	2	2	1	72%	51%	
Sem 1	2012	GSA19_53.jpg	-	130.0	F	5	0	1	1	1	1	1	-	0	1	1	1	1	2	2	1	2	1	1	1	2	1	1	67%	52%	
Sem 1	2012	GSA19_54.jpg	-	130.0	M	5	0	1	1	1	1	1	-	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	78%	49%	
Sem 1	2012	GSA19_55.jpg	-	125.0	F	5	0	1	1	1	1	1	-	0	1	1	1	1	1	1	1	2	1	1	0	1	1	1	78%	53%	
Sem 1	2012	GSA19_56.jpg	-	125.0	M	5	0	1	1	1	1	1	-	0	1	1	1	1	1	1	1	2	2	1	2	1	1	1	72%	51%	
Sem 1	2012	GSA19_57.jpg	-	100.0	F	5	1	1	1	0	0	0	-	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	67%	146%	
Sem 2	2013	GSA19_01.jpg	-	110.0	M	5	1	2	2	2	1	2	-	1	2	2	2	2	1	2	1	3	1	3	2	2	2	2	2	50%	36%
Sem 2	2013	GSA19_02.jpg	-	110.0	F	5	0	2	1	2	1	2	-	1	2	2	2	2	1	1	1	2	1	1	2	1	1	1	1	44%	44%
Sem 2	2013	GSA19_03.jpg	-	115.0	F	5	1	2	1	2	1	2	-	1	2	2	2	2	1	1	2	1	3	1	3	3	1	1	1	61%	34%
Sem 2	2013	GSA19_04.jpg	-	135.0	M	5	2	2	1	2	1	2	-	1	2	3	2	2	2	2	2	1	2	1	2	3	2	2	2	67%	29%
Sem 2	2013	GSA19_05.jpg	-	140.0	M	5	1	2	1	2	1	2	-	1	2	2	2	2	2	2	2	1	2	1	2	2	2	2	2	67%	36%
Sem 2	2013	GSA19_06.jpg	-	105.0	F	5	1	2	1	2	1	2	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	61%	129%
Sem 2	2013	GSA19_07.jpg	-	80.0	U	9	0	1	0	0	0	1	-	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	67%	146%
Sem 2	2013	GSA19_08.jpg	-	80.0	U	9	0	1	0	0	0	1	-	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	67%	146%
Sem 2	2013	GSA19_09.jpg	-	85.0	U	9	0	1	0	0	0	1	-	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	67%	146%
Sem 2	2013	GSA19_10.jpg	-	85.0	U	9	0	1	0	0	0	1	-	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	67%	146%
Sem 2	2013	GSA19_11.jpg	-	85.0	U	9	0	1	0	0	0	1	-	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	67%	146%
Sem 2	2013	GSA19_12.jpg	-	90.0	F	9	0	1	0	0	0	1	-	1	0	1	1	1	1	0	0	0	1	0	0	1	0	1	66%	115%	
Sem 2	2013	GSA19_13.jpg	-	90.0	M	9	0	1	0	1	0	1	-	1	-	-	1	1	1	1	1	1	0	1	1	1	1	1	71%	67%	
Sem 2	2013	GSA19_14.jpg	-	95.0	M	9	0	1	0	1	0	1	-	1	-	-	1	1	1	1	0	0	1	0	0	1	1	1	59%	86%	
Sem 2	2013	GSA19_15.jpg	-	95.0	F	9	0	1	0	1	0	1	-	1	-	-	1	1	1	1	0	0	1	0	1	1	1	1	65%	76%	
Sem 2	2013	GSA19_17.jpg	-	100.0	M	9	1	1	1	1	1	2	-	1	1	2	2	2	1	1	1	1	1	1	1	2	1	1	72%	44%	
Sem 2	2013	GSA19_18.jpg	-	105.0	M	9	0	1	0	2	1	1	-	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	67%	56%	
Sem 2	2013	GSA19_19.jpg	-	105.0	F	9	1	2	1	2	1	2	-	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	67%	36%	
Sem 2	2012	GSA19_21.jpg	-	120.0	F	9	1	2	1	2	1	2	-	1	1	2	2	2	1	1	1	1	2	1	1	3	1	1	61%	43%	
Sem 2	2012	GSA19_22.jpg	-	120.0	M	9	2	2	2	1	1	3	-	1	2	2	3	1	1	2	2	3	2	2	3	2	2	2	2	50%	37%
Sem 2	2012	GSA19_23.jpg	-	125.0	F	9	0	2	0	1	1	2	-	1	1	2	2	2	1	1	1	1	1	3	1	1	3	1	1	56%	63%
Sem 2	2012	GSA19_24.jpg	-	125.0	M	9	1	2	1	2	1	2	-	1	2	2	3	2	2	2	2	1	3	2	2	3	2	2	2	56%	36%
Sem 2	2012	GSA19_25.jpg	-	140.0	F	9	1	2	1	2	1	2	-	1	2	3	3	2	2	2	2	1	3	2	2	3	2	2	2	50%	37%
Sem 2	2012	GSA19_31.jpg	-	140.0	F	9	0	2	1	2	1	2	-	1	1	2	2	2	1	2	1	1	2	2	1	2	2	2	2	50%	43%
Sem 2	2012	GSA19_32.jpg	-	130.0	F	9	1	2	1	2	1	2	-	1	1	2	2	2	2	2	2	1	2	1	2	2	2	2	2	61%	31%
Sem 2	2012	GSA19_16.jpg	-	100.0	F	9	1	1	1	1	1	2	-	1	1	2	2	2	1	1	1	0	2	1	1	2	1	1	67%	45%	
Sem 2	2012	GSA19_20.jpg	-	110.0	F	9	1	2	1	1	1	2	-	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	61%	36%	
Sem 2	2012	GSA19_26.jpg	-	135.0	F	9	1	1	1	2	1	2	-	1	1	2	2	2	2	2	2	1	3	2	2	3	2	2	2	50%	3

Table: ANE_GSA19- Western Ionian. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	6	64.8%	141.1%	0.35
1	32	64.7%	58.4%	0.03
2	17	55.7%	37.3%	-0.31
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	55	61.9%	60.9%	-0.04
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	5	68.9%	159.7%	0.31
1	31	72.8%	44.6%	0.11
2	18	63.6%	30.3%	-0.16
3	1	44.4%	-	-0.67
4	-	-	-	-
5	-	-	-	-
Total	55	68.9%	50.1%	0.03
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	15	59.4%	133.8%	0.44
1	31	62.3%	54.1%	0.13
2	9	54.3%	38.5%	-0.43
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	55	60.2%	73.3%	0.12
Ionian readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	12	69.4%	158.8%	0.31
1	18	82.4%	26.5%	0.10
2	22	69.7%	27.1%	-0.09
3	3	66.7%	21.7%	-0.33
4	-	-	-	-
5	-	-	-	-
Total	55	73.5%	55.3%	0.04

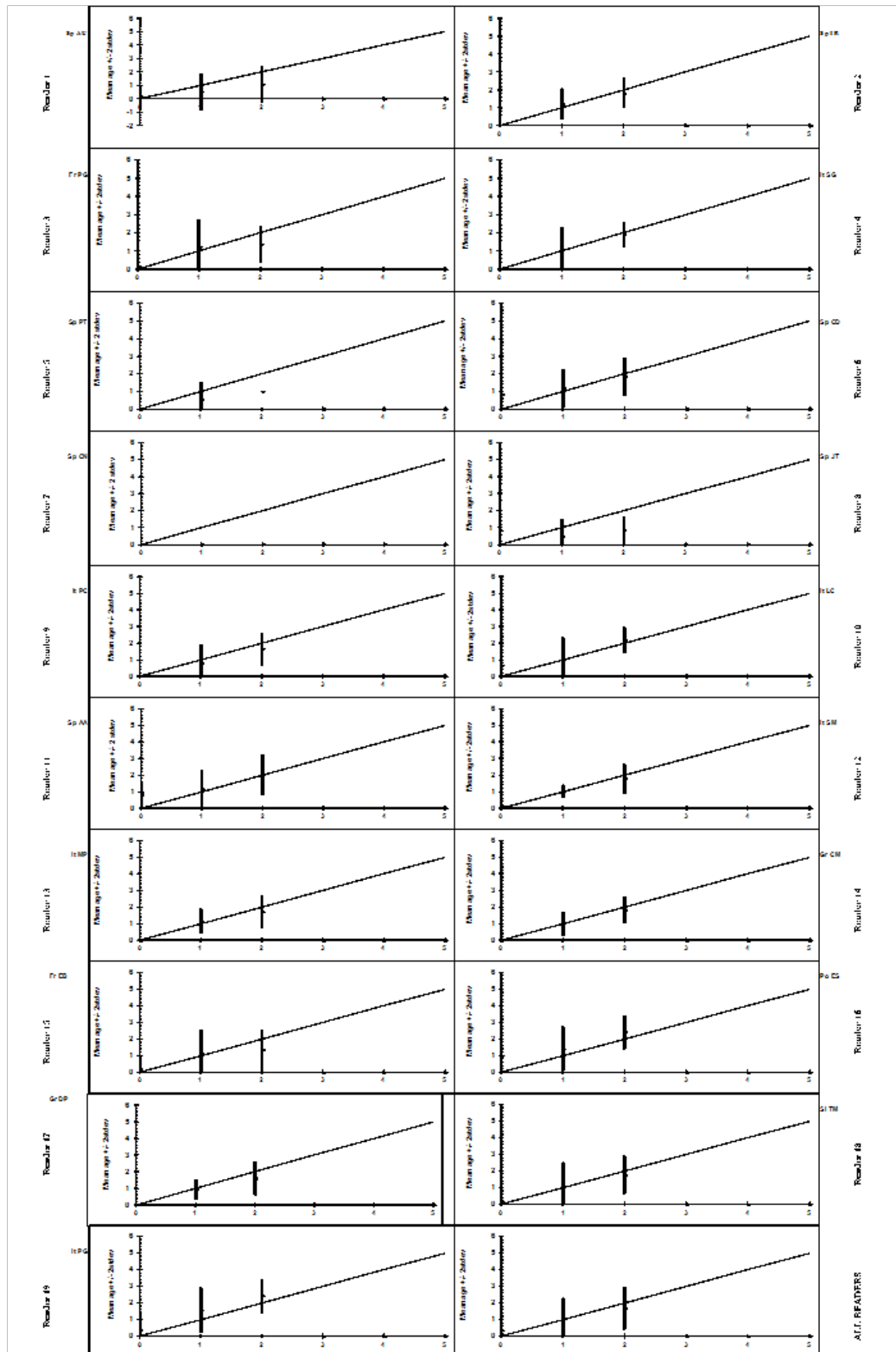


Figure: ANE_GSA19- Western Ionian. Age bias plots with the mean age recorded ± 2 stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

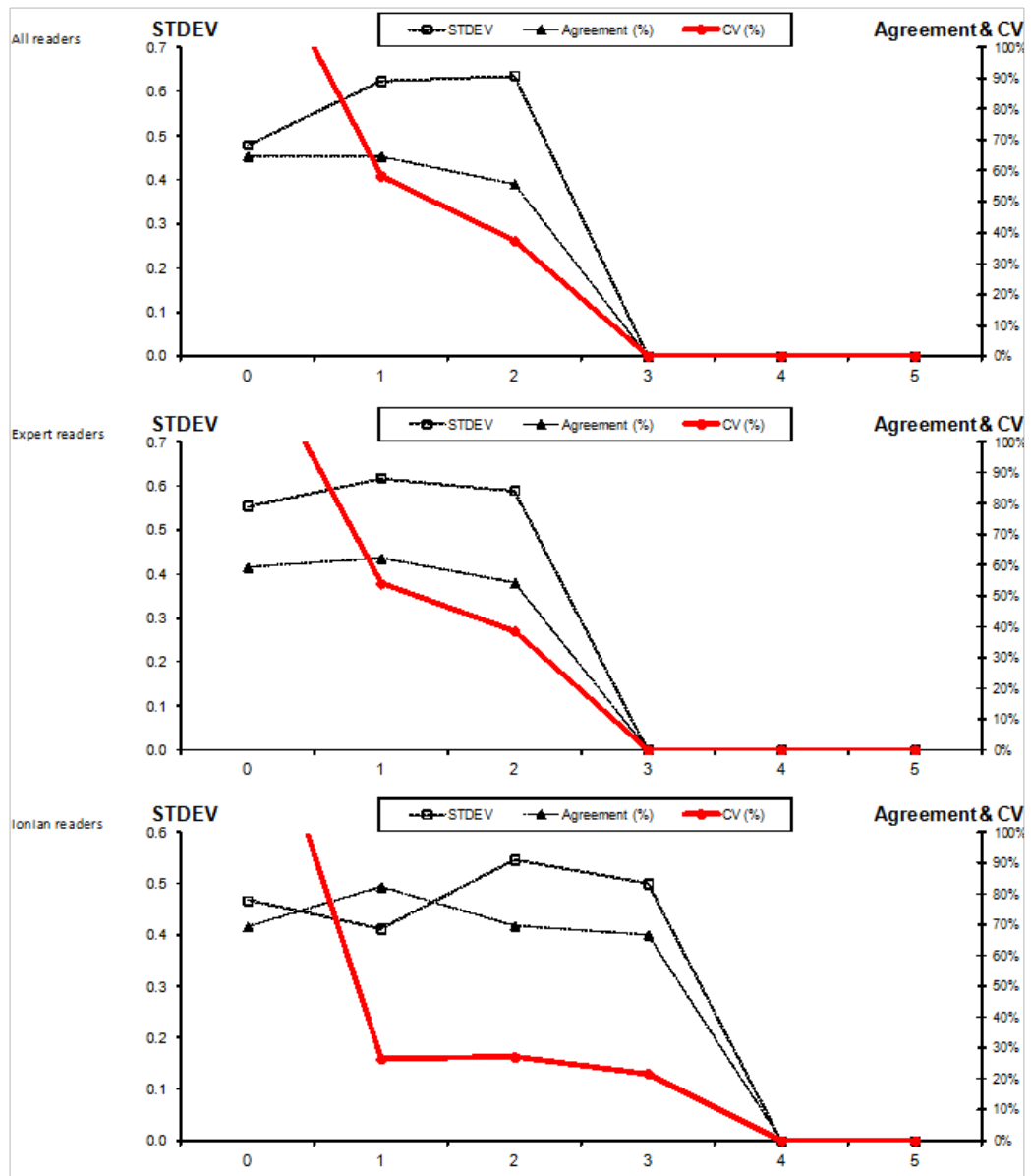


Figure: ANE_GSA19- Western Ionian. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 4.10 ANE_GSA22- Aegean Sea

Table: Age reading results by reader

[illegible]

Table: ANE_GSA22- Aegean Sea. Summary of the average percentage of agreement, CV and relative bias by age.

All readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	7	55.6%	117.4%	0.44
1	62	71.8%	49.0%	0.02
2	1	55.6%	-	-0.22
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	70	70.0%	55.7%	0.06
Intermediate & training readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	20	61.7%	136.7%	0.38
1	49	69.5%	45.7%	0.13
2	1	55.6%	-	-0.22
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	70	67.1%	71.6%	0.20
Expert readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	2	66.7%	150.0%	0.33
1	67	79.0%	39.7%	0.04
2	1	55.6%	-	-0.22
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	70	78.3%	42.8%	0.04
Aegean readers				
Modal Age	Otolith N	% Agreement	CV	Bias
0	26	100.0%	0.0%	0.00
1	43	96.5%	9.9%	-0.03
2	1	50.0%	-	-0.50
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	70	97.1%	6.7%	-0.03

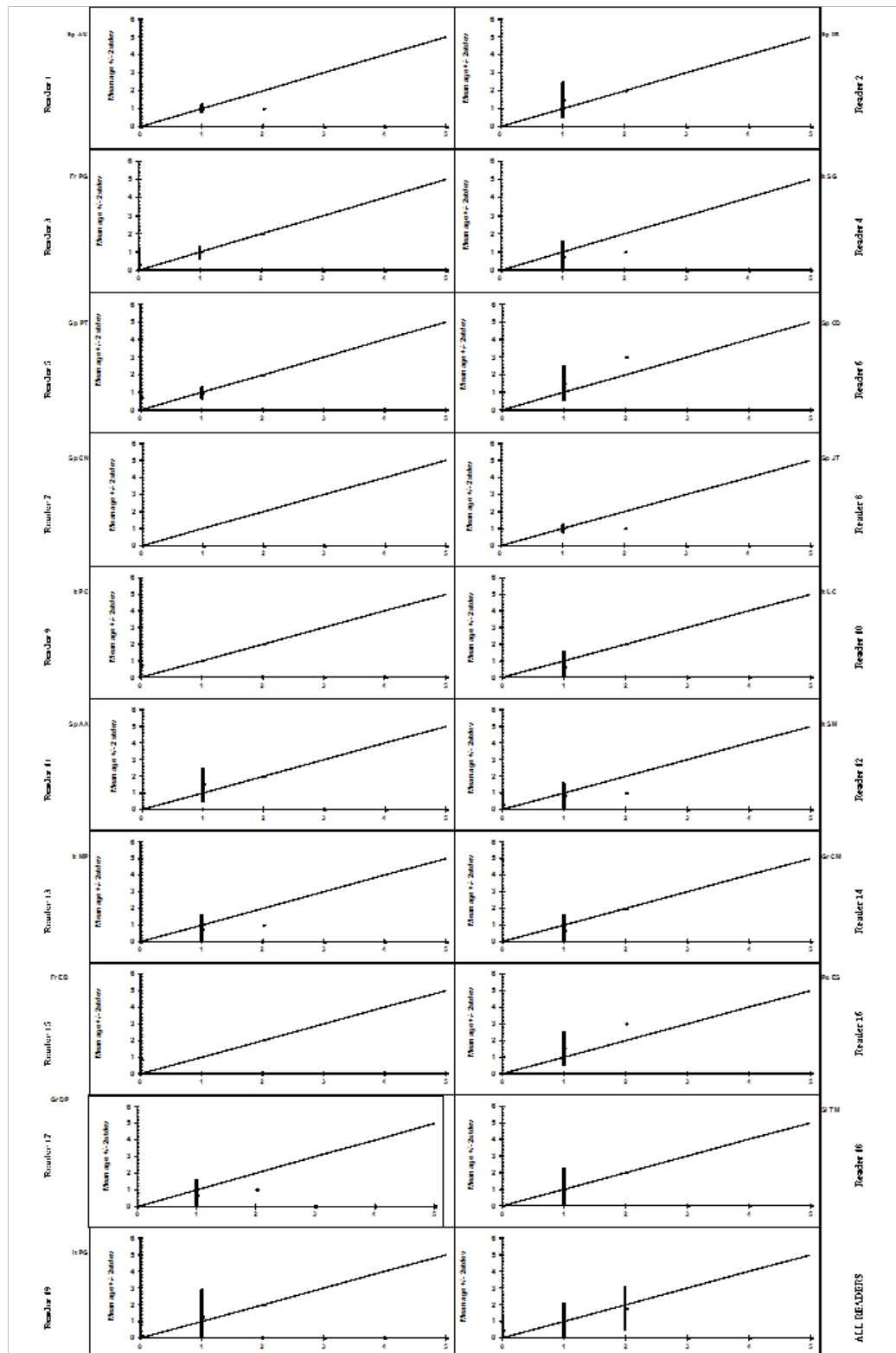


Figure: ANE_GSA22- Aegean Sea. Age bias plots with the mean age recorded \pm 2stdev of each age reader and all readers plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

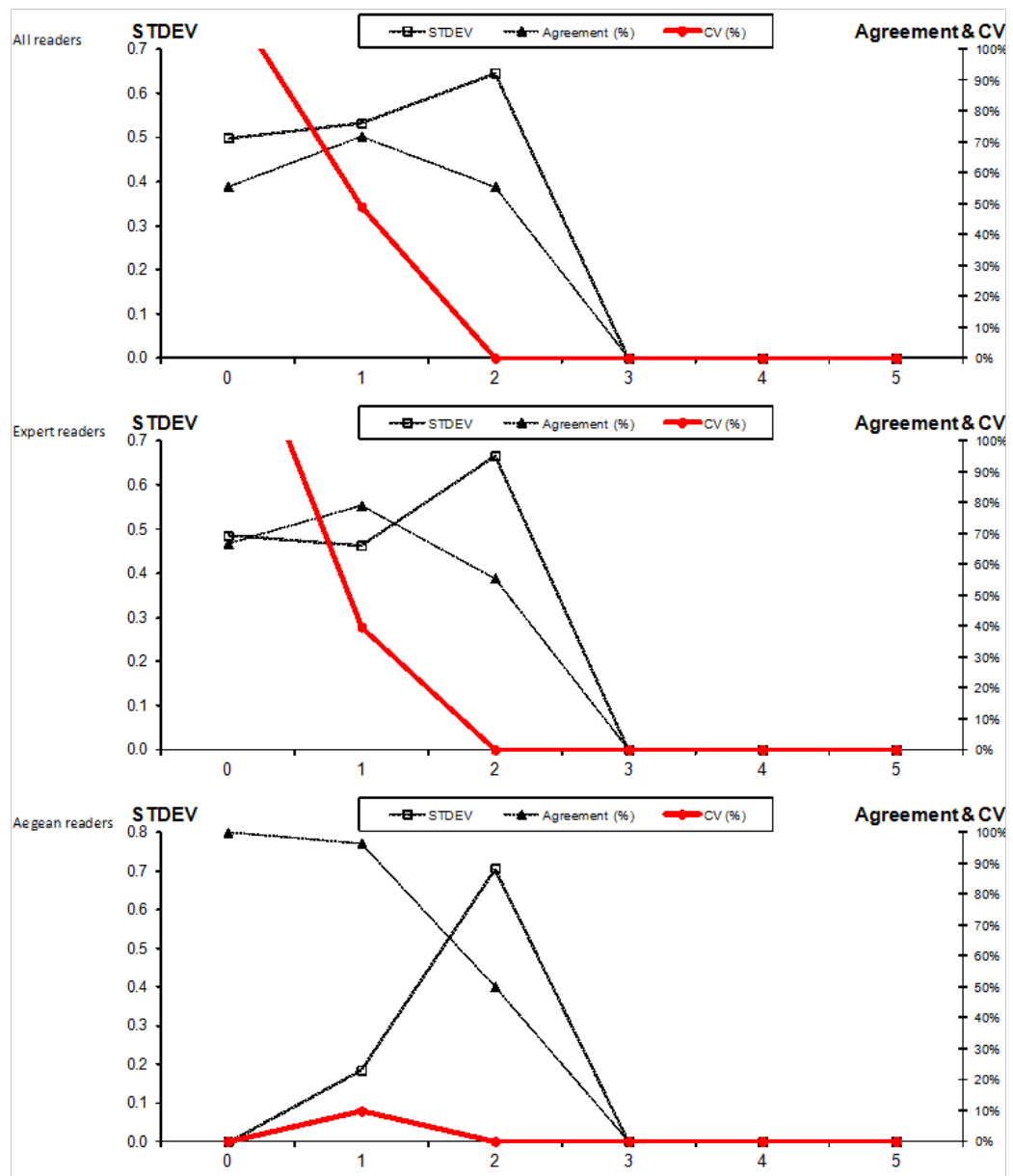


Figure: ANE_GSA22- Aegean Sea. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Annex 5: Contributions to the Workshop. Presentations

During the workshop a total of 20 presentations were performed and they are deposited in the SharePoint of ICES WKARA2. If the reader would be interested in getting access to any of these presentations, they should be requested to the authors.

The list of presentations is the following:

Presentation 1: Begoña Villamor, Andrés Uriarte, Gualtierio Basilone. Review information on anchovy age estimations, otolith exchanges, workshops and validation work done so far. IEO, AZTI, IAMC-CNR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Begoña Villamor

Presentation 2: Uriarte A., Rico I., Villamor B., Duhamel E., Dueñas C., Aldanondo N., Cotano, N. Validation of age determination from Otoliths for Bay of Biscay anchovy. AZTI, IEO, Ifremer. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Andrés Uriarte.

Presentation 3. Hernandez, C., Dueñas-Liaño, C., Antolinez, A. and Villamor, B. Criteria for age determination of anchovy otoliths in Subdivision IXa North: analysis of the rings biometric measures. IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Begoña Villamor.

Presentation 4: Tornero, J. Gulf of Cadiz anchovy otoliths: A preliminary study on the evolution of radius of A1 ring and post-rostrum radius through the year and evolution of the edge trough the season. IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Jorge Tornero.

Presentation 5. Torres, P. Alabran Sea and Western Mediterranean anchovy: Fishery data (catches, lengths, weights and evolution of modal length) and studies of otoliths (microstructure and evolution of edge). IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Pedro Torres.

Presentation 6: Gualtierio Basilone, Salvatore Mangano and Maurizio Pulizzi. Preliminary results on daily growth increment pattern in European anchovy (*Engraulis encrasicolus*) juvenile otoliths in the Strait of Sicily (GSA 16). IAMC-CNR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Gualtierio Basilone.

Presentation 7: Milani, C. and D. Panora. Aegean Sea anchovy: Fishery data and age composition. ELGO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Cristina Milani.

Presentation 8: Ioannis Fytilakos. Aegean Sea and Ionian Sea anchovy. HCMR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Ioannis Fytilakos.

Presentation 9: Adel Gaamour and Sana Khemiri. Tunisian Waters anchovy (GSA 12, GSA13 and GSA14: Biological data and indirect validation studies. INSTM. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Adel Gaamour.

Presentation 10: Begoña Villamor, and Andrés Uriarte. Otolith Exchange Results Of European Anchovy (*Engraulis encrasicolus*) 2014. IEO and AZTI. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Begoña Villamor.

Presentation 11: Begoña Villamor, Andrés Uriarte, Gualtiero Basilone. Problems identification in ageing of otoliths of Anchovy (*Engraulis encrasicolus*) from 2014 Exchange. IEO, AZTI, IAMC-CNR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Andrés Uriarte

Presentation 12: Dueñas- Liaño, C., Hernandez, C., Antolínez, A., and Villamor, B. Problems identification in ageing of otoliths of Anchovy (*Engraulis encrasicolus*) from Divisions VIIIcb and IXa North. IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Clara Dueñas.

Presentation 13: Tornero, J. Problems identification in ageing of otoliths of Anchovy from Gulf of Cadiz (IXa South). IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Jorge Tornero

Presentation 14: Torres, P. Problems identification in ageing of otoliths of Anchovy from Alboran Sea. IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Pedro Torres.

Presentation 15: Gašparevic , D. Problems identification in ageing of otoliths of Anchovy from Adriatic Sea. IOF. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Denis Gašparevic.

Presentation 16: Adel Gaamour and Sana Khemiri. Problems identification in ageing of otoliths of Anchovy from Tunisian waters. INSTM. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Sana Khemiri.

Presentation 17: Ioannis Fytilakos. Problems identification in ageing of otoliths of Anchovy from Ionian Sea. HCMR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Ioannis Fytilakos.

Presentation 18: Cristina Milani. Problems identification in ageing of otoliths of Anchovy from Aegean Sea. ELGO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Cristina Milani.

Presentation 19: Gualtiero Basilone, Salvatore Mangano and Maurizio Pulizzi. Problems identification in ageing of otoliths of Anchovy from Strait of Sicily. IAMC-CNR. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Maurizio Pulizzi.

Presentation 20: Verónica Duque. Problems identification in ageing of otoliths of Anchovy from North of Morocco. IEO. Presentation to WKARA2, Pasaia, Guipuzcoa (Spain), 28 November-2 December, 2016. Presented by Verónica Duque