PELASSES PROJECT REPORT OF THE

WORKSHOP ON ANCHOVY OTOLITHS FROM SUBAREA VIII AND DIVISION IXa.

In AZTI, PASAIA (Spain) from 14 to 18 January 2002

By

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ANCHOVY OTOLITH WORKSHOP:

In AZTI, PASAIA from 14 to 18 January 2002

1- INTRODUCTION:

Within PELASSES project, in subtask 2.3 it was established that at least one workshop will be organized to standardize the age readings of sardine and anchovy. In our February meeting in Lisbon, it was decided that a workshop on anchovy otoliths age reading would be carried out during the rest of the project life, coordinated by AZTI, preferably before summer 2001, although finally this has taken place in January 2002.

The major GOAL of this workshop is to identify major difficulties in age determination and Standardize anchovy otolith ageing criteria for the Bay of Biscay and for division IXa. For the former case AZTI's methodology for age determination was to be presented and discussed by the Workshop in order to decide whether to adopt it as a standard procedure of reference or not.

For the Bay of Biscay two exchange of otoliths took place some years ago, of which results were available at the meeting (Astudillo et al. 1990 & Villamor et al. WD1996).
More recently an exchange of otoliths of the anchovy in IXa (Cadiz) have taken place (Garcia 1998).

For the purposes of this meeting an exchange of otoliths took place during Summer and Autumn 2001 (Uriarte 2002a) based on which precision of current ageing procedures was assessed and served as starting point for analysis and discussions of the workshop. The sets of otoliths examined in the exercise were otoliths arising from the most recent monitoring of the fishery landings and from recent surveys mostly during 2000 and 2001, within the life period of PELASSES. Otoliths older than 3 years did not appear for subarea VIII and ages older than 2 seemed not to appear for subdivision IXa. For the Bay of Biscay the average percentage of agreement across ages and readers (83 %) and the average Coefficient of Variation (CV=30%) were rather low for a three year living fish. The major disagreements arise from the ageing of the oldest age groups (2 and 3). Ages 0 and 1 seem to be much better determined. For the Atlantic coasts and Bay of Cadiz anchovy otoliths a rather similar low precision arisen: The Average percentage of agreement across ages and readers was 84 % and the average CV was 40.8%. A discussion on these results served to introduce the problems on age determination for the different areas during the workshop.

Otoliths in division IXa are known to be rather difficult for age determination. Age reading determination is less established in IXa than for the Bay of Biscay area and therefore standardization of age readings was only tentatively devised and its feasibility was to be discussed during the workshop.

2- ATTENDEES:

1) Uriarte, A. (Coordinator, AZTI) (Reader, only for the Bay of Biscay set of otoliths)
2) Rico, I. (AZTI) (readers nº 1 & 2 had experience in reading anchovies from the bay of Biscay).

3) Cendrero, O. (IEO), (He had experience with reading the bay of Biscay otoliths).
4) Marian Blanco (IEO) (She had experience in age reading of anchovy otoliths)
5) M. Millán (IEO) (She had experience with reading the otoliths from Division IXa south).
6) Morais, A. (IPIMAR) (He had experience with reading the otoliths from Division IXa atlantic shores).
7) Patrick Grellier (IFREMER) (Reader, only for the Bay of Biscay set of otoliths, who had just recently started in reading the otoliths from this area).

Observers: P. Cermeño (AZTI), B. Beldarrain (AZTI).

See complete addresses of attendees in Annex 1. Further details about reader's experience in otolith age reading was described in the Exchange programme report (Uriarte 2002a).

3- OBJECTIVES:

Objectives concerning the Bay of Biscay anchovy:

1- Analyse the results concerning age precision of the exchange programme on anchovy otoliths from subarea VIII and identify major difficulties in age determination concerning observed disagreements.
2- Present and discuss AZTI's methodology for ageing anchovy otoliths from the Bay of Biscay.
3- Discuss and agreed on protocols for Ageing determination criteria.
4- Evaluate improvements in ageing precision among institutes by the end of the meeting.
5- Establish a digitalized agreed collection of otoliths for the Bay of Biscay anchovy.
6- Establish an agenda of work for continuous tracing of quality in anchovy age determinations for the Bay of Biscay area
7- Future research.

Concerning Anchovy in IXa

8- Analyse the results concerning age precision of the exchange programme on anchovy otoliths from division IXa and Identify major difficulties in age determination concerning observed disagreements.
9- Discuss possibilities of agreeing on protocols for Ageing determination criteria.
10- Establish a digitalized agreed collection of otoliths for anchovy in division IXa?
11- Establish an agenda of work for continuous tracing of quality in anchovy age determinations for the area IXa.
12- Future Research

4- MATERIAL AND METHODS:

The workshop developed through discussions of the exchange programme results and on common examination and discussion of the interpretation given to the otoliths of the exchange (see Annex 2). The sets of otoliths prepared for the Exchange otolith programme organised before served as starting point for discussion and further reading during the workshop meeting either for the Bay of Biscay anchovy as for anchovy in Division IXa, in order to check improvements in consistency during the meeting.

SETS OF OTOLITHS:

SET A) 200 OTOLITHS FROM THE BAY OF BISCAY, assuring all range of lengths (and hence ages).

- 100 otoliths from the first half of the year:
  IEO supply 60 from Surveys in 2000+01 and AZTI 40 from fishery 99/00.
- 100 otoliths from the summer autumn fisheries (99/00)
  IEO supply 30 , AZTI 30 and IFREMER 40 (in the latter case only from 1999)

SET B) 240 OTOLITHS FROM AREA IXa:

- 110 otoliths from West Portuguese coasts (IXa central) arising from surveys in 2000 and 2001, prepared by IPIMAR: modifying Alexander's proposal:
  WEST COAST (PELASSES Mars2001) – **60** (length range: 8-17.5)
  WEST COAST (NOVEMBER 2000) – **50** (length range: 10-19)
- 130 otoliths from IXa south arising:
  IPIMAR: ALGARVE (PELASSES 2001Mars) – **30** (length range: 11.9-14.5).
  IEO fishery in Bay of Cadiz (IXa south). –**100**:
  (50 from the first half of the year and 50 from the second half).

In addition a set of 75 simple (easy to read) otoliths from the bay of Biscay anchovy of spring 1995 were examined during the meeting (coming from samples taken in April –95042828- and May –95050230).

PREPARATION OF THE SETS OF OTOLITHS:
As agreed in previous exchanges and directly among readers of anchovy otoliths, these otoliths are mounted entire within Eukit on black slides of 10 pairs of otoliths each. Otoliths are mounted with the sulkus facing down.

AGE DETERMINATION PROCEDURES

A proper discussion (and revision for the Bay of Biscay) of the procedures for age determination was a goal of the workshop itself. Nevertheless for the exchange programme as well as for the workshop the Minimum knowledge for age determination was:

a) Conventional birth dates for increasing in one year the age of an anchovy, when trespassing that date, is 1st of January.
b) Spawning time is usually in spring (or secondarily in Autumn in IXa) and maximum growth in spring and summer.
c) True Annual rings will be those formed in winter each year. Other rings may be present or appear throughout the year and cause problems in age determination (checks).

For the age reading exercises, each reader received forms to be fulfilled with the following information for each selected otolith:

- Sample and Slide identification code where the otolith is contained
- Month of capture
- Length, weight and sex of the fish.

In the forms, each reader indicated:

- the age assigned to each otolith
- otolith edge (hyaline –H- or opaque –O-),
- reliability of age determination: 0-sure, 1- doubtful and 2-very doubtful or difficult.

Although the otoliths had already been aged before the workshop in the exchange programme, the new readings were made without looking at the previous age readings performed in the past.

All data were analysed using the Workbook Age Reading comparisons of Eltink (2000) and following the recommendations of the Guidelines and tools for age reading comparisons (Eltink et al. 2000)

5 RESULTS

5. 1 RESULTS ON OTOLITHS FROM THE BAY OF BISCAY:

5.1.1 Difficulties in ageing this anchovy according to the Exchange results:

The exchange results revealed a poor level of precision (CV 30%) and agreements among readers (APE of 83%). Most of the problems came from the age determination of the oldest age groups (2 and 3), particularly for the IEO and IFREMER readers which are relevant institutes for the monitoring of fishery on the Bay of Biscay anchovy. This conclusion was deduced by comparison of their age reading with the modal ages for each otolith. The IEO readers relative to the modal age tended to underestimate the oldest age groups, implying younger age compositions than the ones produced by the rest of participants. This Institute monitors about 12% of the International landings of anchovy. IFREMER , that accounts for about 48% of the International landings produced a bit more age 3 than expected. AZTI that monitors about 40% showed the highest agreement with the modal ageing followed by the reader from Portugal (which is not implied in the monitoring of this fishery).

A general discussion about the reasons that might explain the agreements and discrepancies appearing in the exchange report was made at the beginning of the workshop leading to identify three major reasons for disagreements:

a) insufficient typical annual growth pattern recognition
b) insufficient criteria about the otolith edge that can be expected to be seen along the year
c) difficulties in differentiating between true annual rings and false rings (or checks).

See several examples in the exchange report (Uriarte 2002a). The major reason for these problems is the lack of any published validated ageing methodology of otolith age reading for the bay of Biscay anchovy.
Subsequent to this discussion a description about the major pattern of annual growth (a), edge formation along the year (b) and concerning typical checks present in the otoliths of the Bay of Biscay anchovy was presented along with the procedure for age determination followed at AZTI.

5.1.2 Ageing methodology of anchovy otoliths followed at AZTI

The procedure followed at AZTI for age determination based on anchovy otolith examinations was presented along with the validation of this procedure (Uriarte 2002b ms. AZTI interim report). This was made in order to discuss it and decide whether to adopt it as a procedure of reference for the institutes or not. Given the fact that AZTI's descriptive method for age determination is currently in Spanish here below follows a summary of that method and its validation:

AZTI's method is based on the 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.

- Typical annual growth of the otoliths is established, by which growth during the first, second and third years of life (corresponding to 0, 1 and 2 years old groups) diminish to about ½ or 1/3 of the growth performed during the previous year of life. Older ages (4 and 5 years old present a rather similar growth to the one experienced at age 3). **Figure 5.1.2.1** and **5.1.2.2** present typical otoliths at different ages in spring time showing the typical annual growth described above.
- Seasonal formation of the otolith edge follows that of temperate fishes in the northern hemisphere with maximum otolith growth (opaque white band formation) in summer months, and growth detentions (with hyaline rings) in winter time. However the starting of the white edge during spring time changes with ages, being remarkably sooner at age 1 than at older ages (**Figure 5.1.2.3**). As a result of this, in spring 1 year old anchovy have typically already started the deposition of the opaque growth band, whereas 2 years old or older fishes have mostly hyaline edges (or at the end of the spring in early formation of the opaque band) (**Figure 5.1.2.1)**.
- Typical checks occur before and after the first winter ring is formed, during age 0 and age 1 of this anchovy. The most typical one is that formed during June/July in many of the one years old anchovy at the peak of their first spawning period, which is considered to be a spawning check (**Figure 5.1.2.4**). Not all the years, 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, their position will often differ from the expected position of the true annual rings. True annual ring usually form stronger discontinuities in the otolith structure than checks. In addition, the prior knowledge of the most typical expected checks will serve to more easily recognize a check. And finally, when doubts occur its assumption should allow for a better fitting to the typical annual (and seasonal marginal) growth of the otolith than in the alternative of assuming that it is a true annual ring.

Age determination procedure based on the examination of the otoliths of the Bay of Biscay anchovy is obtained according to the above knowledge of growth pattern of anchovy otoliths and knowledge of the date of capture, so that the following two criteria are satisfied:

a. Criteria of complete growth zones in conformity with the typical annual growth pattern: Age equals the number of complete opaque growth zones corresponding to the expected annual growth pattern of the otoliths and excluding the marginal edge development of the year. In case the number of opaque zones do not correspond with the typical expected annual growth pattern the existence of some checks can be suspected and evaluated.
b. Criteria of the edge in conformity with the expected seasonal edge growth by age: If the edge of the otolith do not correspond with the expected otolith edge of the age derived from above (a) criteria, then alternative interpretations should be considered (such as presence of checks). This may be relevant for instance to differentiate between ages 1 and older during the first half of the year, etc. In those cases a decision can be taken about the most likely age of the fish or alternatively the otolith can be rejected for age determinations.

Validation of the AZTI's ageing method is achieved by two indirect methods:

a) Following the growth of very strong cohorts in the length composition of fishery landings and in direct Biomass estimates achieved through the daily egg production method; showing its correspondence with otolith structures for the 1982, 1989 and other cohorts and

b) Following the marginal otolith structure development throughout the year.

Never before a full validated description of ageing procedures for the Bay of Biscay had been documented or established. Elaboration of a CD for diffusion of this document and with didactic photo files of successive otoliths for selected cohorts across several years is foreseen to be accomplished during 2002.

The workshop members considered that the validation presented was convincible enough as to adopt the proposed methodology as a valuable guidelines for the age determination of the bay of Biscay anchovy otoliths. It was decided to adopt it as a standard methodology of reference to be followed in future by the institutes. This will constitute an initial standard for ageing till further research may refine or modify the proposed methodology.

5.1.3 Interim exercise: Bay of Biscay anchovy Workshop calibration.

Reading of an easy set of Spring otoliths: In order to fix the basic annual growth pattern a set of 75 simple (easy to read) otoliths of the bay of Biscay anchovy from spring 1995 (with ages ranging from 1 to 4) were examined during the meeting. The set of otoliths and the Summary results are presented in Table 5.1.3.1, 3.2 & 3.3. The average level of agreement was 91 % and the Coefficient of Variation (CV) was of only 9 %. These results were clearly better than those obtained for the exchange exercise revealing the easier nature of the otoliths and perhaps a better comprehension by the readers of the expected annual growth bands for each age class.

5.1.4 Checking Improvements: Second reading of exchange otoliths.

A second reading of the otoliths included in the exchange exercise was performed during the workshop trying to implement the methodology proposed above. The results follow:

Table 5.1.4.1 details the length, sex and month of landing of the set of otoliths selected for the exchange programme from the Bay of Biscay region (set A) along with the ageing produced by each reader. The last two columns give the Modal age, the percent of agreement relative to Modal age and the Precision of reading as the Coefficient of Variation in relation to the average age. The Average percentage of agreement across all ages and readers is 92.5 % and the average CV equals 11.4 %. Clearly this results imply a noticeable improvement in age consistency and precision in comparison with the exchange results (that gave APE of 83 % and an average CV of 30%).

Table 5.1.4.2 shows that almost all otoliths were read by the participants (first sub-table of Table **5.1.4**.**2**). CV is minimum at age 0 (see also **Figure 5.1.4.1**) and a bit higher at older ages as the percentage of agreement diminishes with age. However these levels of CV (of about 10-12%) are four times smaller than those of the exchange exercise, and the level of agreements (about 90%) suppose a remarkable improvement over the exchange results concerning the older ages (which were about 70% and 56% for ages 2 & 3 respectively). The sub-table of relative bias indicates a slight underestimation by –0.14 of age 3 and smaller for the others (while the underestimate at age 3 was of –0.66 in the exchange exercise). **Figures 5.1.4.2 & 3** also show that the amount of bias is rather low. Nevertheless, despite the general improvement in the consistency and precision of ageing anchovy otoliths, some readers still deviated significantly from the modal age and particularly readers 3, 4 and 5 (from IEO) (**Table 5.1.4.3** bottom table). These readers also showed significant deviates in the exchange exercise, but if then they tended to give less ages 2 and 3 than the modal reading, now they have corrected it, but reader 3 and 4 have moved to the opposite situation giving instead more ages 2 than the rest of readers (**Table 5.1.4.3**). Nevertheless the magnitude of the bias as shown in **Figure 5.1.4.4** by readers is certainly now rather low in comparison with the exchange results. Best readers in relation to Modal age appear to be AZTI readers who have the longest experience in reading this otoliths.

Tables 5.1.4.4 and **5** show that the degree of agreements do not change markedly between months but mainly among ages. However it seems that the difficulty of reading increases during the second half of the year (Semestre 2 strata).

In **Table 5.1.4.3** as in **Figure 5.1.4.6** it is shown in general mean length at age increases with age, except for a few exceptions at age 0 of some readers.

5.1.5 Discussion: unsolved problems and future quality assurance and research.
The general pattern of development of the anchovy otoliths in the Bay of Biscay and general rules and guidelines for age determination were adopted according to AZTI's descriptive work (Uriarte 2002b, ms.) and summarised in **section 5.1.2.**

Certainly the agreement achieved during the workshop on following AZTI's procedures for the ageing of bay of Biscay anchovy otoliths has lead to a noticeable improvement in consistency and precision of age determinations in comparison with the results of the otolith exchange programme. Most of the typical and most common otoliths were well classified by most of the readers after the discussions and interim exercise during the

workshop. Most of the otoliths presented in the exchange report are better agreed now and explained here below:
Figures 5.1.5.1 and **2** present two otoliths corresponding to 1 year old anchovies, showing the same type of opaque growing edge at early spring, however the second one shows an internal check which in case of being taken as a true winter ring may lead the reader to assign the age of 3. In this case the criteria of the expected edge helps in determining the nature of the inner ring. **Figures 5.1.5.3** and **4** present the cases of 2 years old otoliths showing spawning checks: in the first case the check is weaker than in the second one. In the former case the check is at a conflictive position reminding the expected position of the second annual ring and hence causing doubts about its true nature. In the second case the check is placed midway between the first winter ring and the hyaline border which is in disagreement with the expected decreasing annual bands of growth for ages 2 and 3 and hence suggesting to be a check. Both otoliths are very similar and interpretation of the weak rings previous to the edge as spawning checks leads to recognise the typical expected growth for 2 years old anchovies. **Figure 5.1.5.5** present the case of a typical 3 year old anchovy in Spring which was conflictive during the exchange programme (with only 57 % of agreements) but for which the criteria of the expected annual growth pattern and that of the edge leads clearly to interpret it as corresponding to a 3 years old anchovy (the workshop agreement scored to 86%).
There were still several difficult otoliths for age determination which could not be agreed among readers, most of them because partially diverged from the usual growth pattern and hence are difficult to be coupled or assimilate with the general guidelines for age determination proposed by AZTI. For instance **Figure 5.1.5.6** shows a case of a doubtful 1 or 2 years old anchovy otolith from summer time: Modal interpretation assign an age 1 but with a strongly marked spawning check what makes it hard to be interpreted as a check; the alternative being interpreting it as the second winter annual ring followed by the current year growth (but in this case the arising second year annual growth would be rather small in comparison with the expected one). This conflict (well marked ring in a bad position) lead to a bad level of agreement among readers even during the workshop (of only 57% with the modal age).

Major difficulties still present at the end of the workshop seem to refer to discriminating between true winter rings from checks, as exemplified above. To this point the prior knowledge of the expected annual growth and of the most frequent and typical checks, as well as the individual experience of each reader is very valuable. When doubts occur, the criteria of complete growth zones in conformity with the typical annual growth pattern should be helpful: if the doubtful rings are checks then their assumption should allow for a better fitting to the typical annual (and seasonal expected marginal) growth of the otolith than in the alternative of assuming that they are true annual rings.

Assurance of future quality:
It was admitted by all participants the convenience of assuring that the current levels of precision among readers are maintained (if not improved) as much as possible in future. To this purpose the following conclusions and recommendations were adopted:

- to adopt AZTI's procedures for age determination (Uriarte 2002b, ms.) as a standard methodology of reference to be followed in future by the institutes, until further refinements or modifications improve that methodology.

- To establish a digitalized validated collection of otoliths for the Bay of Biscay anchovy from the set of photos available from the work of validation followed in AZTI with the 1982 and 1983 and 1989 cohorts. This can be used as material for training of future readers and reminding for the current readers.
- To collect in a (freely distributed) CD the validation report, the methodology for age reading and the digitalized validated collection of otoliths for the Bay of Biscay anchovy. This will be produced by AZTI during 2002.
- To continue with some informal exchanges of otoliths among readers for them to keep in contact and discuss the difficult otoliths: AZTI is open during an interim period of about two years to read the otoliths of anchovy from IEO and, while the new readers become familiar with the ageing procedures.
- To suggest for the organization of a new exchange programme in 3 years (in 2005), to check the consistency and precision of age readings by then in comparison with the current ones. The organization of that programme could be discussed within the frame of ICES by the members of the Mackerel, Horse mackerel, sardine and anchovy working group.

Topics for further research: Selecting some well agreed otoliths or particularly problematic ones and read the daily rings as a way for resolving the badly age allocated otoliths.

5. 2 RESULTS ON OTOLITHS FROM Subdivision IXa:

5.2.1 Difficulties in ageing this anchovy according to the Exchange results:

The exchange results revealed a poor level of precision (CV 40.8%) and agreements among readers (APE of 84%). In principle only ages ranging from 0 to 2 appeared and hence those results are rather poor for a two year living fish, although similar to the levels achieved for the Bay of Biscay anchovy in the exchange programme. The major disagreements arose from the ageing of the oldest age groups (age 2, which showed an APE of 68%). Age 0 (white clean otoliths as those from the Bay of Biscay) and age 1 (Figure 5.8) seemed to be better determined (Average APE of about 88%).

The otoliths originating from Portuguese coasts, in IXa central and central-north, were partly different from those of the Algarve and Bay of Cadiz. The former had more often poorly marked annuli than the latter's. Algarve and Bay of Cadiz otoliths were rather similar, with more or less pronounced annual rings a bit less intense than in the Bay of Biscay but with a general growth band pattern more similar to the latter area than those from IXa central-north and central-south

Those who posed the major amount of problems were mainly due to poorly marked annuli or almost no clear annuli till the edge. In those cases the nature of the edge was considered of relevance to undertake a decision for the age determination, and since no clear rules are established for this area the amount of discrepancy among readers increased in those cases.

Although the degree of agreements did not change markedly between months but mainly among ages, the difficulty of reading correctly age 1 may higher during spring at least for the otoliths available from Division IXa south.

A general discussion about the reasons that might explain the agreements and discrepancies appearing in the exchange report was made at the beginning of the workshop leading to identify several major reasons for disagreements:

a) insufficient typical annual growth pattern recognition
b) insufficient criteria about the otolith edge that can be expected to be seen along the year
c) difficulties in differentiating between true annual rings and false rings (or checks).
d) Badly marked first annual rings in some cases, as for example in cases of the Division IXa central and central-north.
e) Possibility of mixing of several cohorts through the year arising from separate spawning periods in spring and autumn. These cohorts may lead to different structures of otoliths, as for instance badly marked first annual ring.

The three first reasons are similar to those mentioned for the Bay of Biscay area, but the others are of particular relevance in Division IXa. Several examples of agreements and disagreements are presented in the otolith exchange report (Uriarte 2002a). There is no validated ageing methodology of otolith age reading for Division IXa and this made difficult a proper discussion of the alternative age readings available, however some general consensus was attained by the attendees to the workshop which can be helpful as starting guidelines for ageing these otoliths (see next section).

5.2.2. Agreement on general guidelines for Basic ageing methodology:

Basics for ageing methodology of anchovy otoliths from Division IXa:
Following the discussions made in the workshop it was considered that the basics of growth pattern of the otolith and ageing methodology for otoliths from this Division could be similar to those described for the Bay of Biscay anchovy before. Several examples of rather typical otoliths that can be assimilated to that growth pattern appear in **Figures 5.2.2.1, 2.2, 2.3** and **2.4** for ages 1, 2, and 3 respectively (the latter as interpreted by the first time during the workshop discussions).

However some particularities in this area are suspected to require further developing of the former general rules, as follows:

- There are some 0 group anchovies entering the winter period with very small sizes that may not lay their first winter annual ring (A1); in those cases ages 1 would too vaguely show any winter ring (see example in **Figure 5.2.2.5**). It is a common procedure during the first half of the year to assign the age of 1 to all otoliths not showing any ring, because it would be very unlikely for a born in the year to show such a fast growing as to become recruited to the fishery during the first half of the year. However, during the second half of the year they could be unduly assigned to age 0. This situation causes the problem of discriminating true age 0 (born in the year) from false age 0 (born the year before but not showing any remarkable ring).
- These one year old fishes, with none or poorly marked first winter ring, may lay down during the second winter a strong hyaline ring, that can be used as a clue

for assigning them since then onwards the age of two or more years old, and hence the first year ring can be searched or guess in the rather opaque white (first year and a half) growth band (see example in **Figures 5.2.2.6, 7 & 8** of otoliths interpreted as ages 2 under those conditions, and in **Figure 5.2.2.9** of a likely age 3). In some cases the general looking of the otoliths of these fishes may help to suspect about its older age than annuli seen. In general the criteria of the type of expected edge for each age group along the year can be the most firm basis for its correct allocation to an age. These cases occurred in either Subdivision IXa central north as in Bay of Cadiz. During the second half of the year, those 1 year old anchovies not showing any first winter ring might be also recognisable by the sexual gonad development, since all one years old may (by comparison with anchovies in other temperate regions) attain full maturity during its first year of life.

- From another point of view, there might be some true 0 groups entering already the fishery at the end of the first half of the year, that could already be assigned to the 0 group. This factor suggest that a revision of the conventional date for starting accepting the existence of the 0 groups can be considered, such as starting from May or June that would help putting together consistently the same cohorts.

5.2.3 Checking Improvements: Second reading of the otoliths from the Bay of Cadiz.
A second reading of part of the otoliths included in the exchange exercise was performed during the workshop trying to implement the general guidelines of age reading proposed above for this area (with their particularities). Because of the limited time available only otoliths from the Bay of Cadiz were read again during the workshop, they suppose half of the total otoliths from set B analysed during the exchange programme. They were preferred over the Atlantic shore otoliths because in the Bay of Cadiz it takes place the major fishery on anchovy in Division IXa. Here, as in the exchange programme for set B, readers 2 and 7 did not participated so that a total of 5 readers took part in this exercise during the workshop.
The results follow:

Table 5.2.3.1 gives the details for each otolith selected from the Bay of Cadiz in Subdivision IXa south, along with the age determination produced by each reader: The Average percentage of agreement across all ages and readers is 88 % and the average CV equals 26%. These results suppose an improvement in precision compared to the levels achieved during the exchange programme for this regions, which were 78.4% for APE and 47.2% for CV.

Table 5.2.3.2 shows the amount of otoliths read by the participants (first sub-table). Whilst during the exchange programme there was no determination of age 3, now a few otoliths were aged 3; so total ages ranged from 0 to 3. CV is minimum at age 2 (11%), it reaches about 20 % for ages 1 and 3 and it is maximum at age 0 (see also **Figure 5.2.3.1**). However the percentage of agreement for ages 1 and 2 are similarly (about 88 %). In comparison with the exchange results the CVs for ages 1 and 2 have been halved and the APE is notable increased (by more than 10%) for ages 1 and 2, although it remain similar for age 0.

The relative bias are about +/–0.08 for ages 0-2; this supposes for age 2 a strong reduction compared to the exchange programme (where it had –0.3) (see also **Figure 5.2.3.2 & 3**). The few age 3 have the lowest precision and APE. Reader 5 (the one working with the Bay of Cadiz) has become the most consistent reader with the modal ages and it is the first place of the overall ranking (see also **Figure 5.2.3.4**). **Table 5.2.3.3** shows the age composition of this set of otoliths and the general level of agreement among readers, which has become in general statistically compatible among them (while this was not the case in the exchange results). In this table mean lengths at age are also shown, with a natural progression in length at age for all readers.

Tables 5.2.3.4 shows that the degree of agreements do not change markedly among months.

5.2.4 Discussion: unsolved problems and future quality assurance and research.

The adoption for the otoliths from the Bay of Cadiz of the general rules for age determination of anchovy otoliths followed in the Bay of Biscay (adapted with the particularities mentioned before) have resulted in an increased of about 10% in the level of agreement among participants (to 88%) and in an improvement in the precision of the age determinations (halving the CV resulting during the exchange exercise). The major improvement has been attained with age 2, showing a strong increased in APE from 70% to 88% and a large decrease in average bias from –0.3 to -0.08 and in CV from 29.8% to 11.6%. In addition the discussion performed during the workshop has lead to the realizing the presence of some fishes of age 3; which is the first time they appear in the age composition of these anchovy catches. Similarly age 2 might be more abundant than previously detected. This seems to indicate that a revision of the age determination performed during the last years would be advisable.

Despite the benefit of adopting as a starting unifying criteria the guidelines for the Bay of Biscay otoliths, the presence of checks and strong differences in the growth pattern of different fishes make certainly difficult the interpretation of these otoliths. For instance **Figures 5.2.4.1** and **2** present difficult otoliths for ages 1 and 2 showing checks and small second year growth band respectively. Given the current uncertainties, the information contained in the length of the individuals is considered so far helpful to judge the general suitability of the suspected age.

In summary, the complexity of otoliths from Division IXa seems to be higher than in the Bay of Biscay and this situation deserves further research in order to develop a standard *ad hoc* methodology for determining the age of this anchovy population as suggested below.

Further research is needed on the otoliths from Division IXa in order to overcome the difficulties pointed out during the exchange and the workshop. The ones suggested were:
a) Following of length mode cohorts and otolith development of the edge throughout the year and for several years (if possible) to establish a standard *ad hoc* methodology for determining the age of this anchovy population through otolith examination.
b) Selecting some well agreed otoliths or particularly problematic ones and read the daily rings as a way for resolving the badly age allocated otoliths.

It was admitted by all participants the convenience of assuring that the current levels of precision among readers are maintained (if not improved) as much as possible in future. To this purpose the following conclusions and recommendations were adopted:

- To adopt for Division IXa (with some modifications) the basic ageing methodology followed in AZTI for the bay of Biscay anchovy for age determination, in an interim manner, until proper standard methodology *ad hoc* for this anchovy population is established. The modification mentioned above refer to admitting for the otoliths from Division IXa, the existence of several fishes with none or poorly marked first winter ring (as discussed in section 5.2.2).
- To suggest the organization of a new exchange programme and a workshop in 3 years (in 2005), to review the advances in establishing a proper methodology for age reading and the consistency and precision of age readings by then in comparison with the current ones. The organization of that programme could be discussed within the frame of ICES by the members of the Mackerel, Horse mackerel, sardine and anchovy working group.

It was considered that the current stage of standardization of age reading methodology was too preliminary as to establish a digitalized validated collection of otoliths for the anchovy in Division IXa.

CONCLUSIONS OF THE WORKSHOP

- For the Bay of Biscay area (Division VIII) the standard procedure followed at AZTI for age reading of anchovy otoliths (Uriarte 2002b) has been proposed and adopted. A CD with the validation, the method and a large set of didactic validated photos produced at AZTI will be delivered to every participant of the workshop.
- For the otoliths from Division IXa the workshop has recommended following the general rules for age determination of anchovy otoliths applied in the Bay of Biscay (adapted with some particularities of the Division IXa, mainly that of the existence of several fishes with none or poorly marked first winter ring).
- The discussion and the adoption of the above guidelines during the workshop has lead to a general improvement in the agreement among readers on age determinations for the Bay of Biscay and Division IXa anchovy otoliths, till about 92 and 88 % of agreement respectively, in comparison with the exchange programme; which supposes an improvement of about 10% in both cases. The average Coefficients of variation were by the end of the workshop 11.4% and 26% for the Bay of Biscay or Bay of Cadiz otoliths.
- Assurance of future quality is being devised by free collaboration and exchange among the institutes with routinary samples. In the case of the Bay of Biscay assurance of future quality it is to be supported by a digitalized validated collection of otoliths, and publication of the validation report and the methodology for age reading (They all being included in the CD mentioned above).
- The workshop suggest the organization of a new exchange programme in 3 years (in 2005), to check the consistency and precision of age readings by then in comparison with the current ones. The organization of that programme could be discussed

within the frame of ICES by the members of the Mackerel, Horse mackerel, sardine and anchovy working group.

- Further topics of research:
    - General recommendation: Selecting some well agreed otoliths or particularly problematic ones and read the daily rings as a way for resolving the badly age allocated otoliths.
    - For division IXa: following of length mode cohorts and otolith development of the edge throughout the year (and for several years if possible) is advisable.

REFERENCES:

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

Eltink, A.T.G.W. 2000. Age reading comparisons. (MS Excel workbook version 1.0 October 2000) *Internet: http://www.efan.no*

Eltink, A.T.G.W., A.W. Newton, C. Morgado, M.T.G. Santamaria and J. Modin, 2000: GUIDELINES AND TOOLS FOR AGE READING. (PDF document version 1.0 October 2000) *Internet: http://www.efan.no*

GARCIA SANTAMARÍA, T., 1998: Anchovy (Engraulis enceasicolus L.) otolith exchange. EFAN Report 4 -98.

URIARTE A.2002a: 2001 anchovy otolith exchange programme from subarea VIII and Division IXa (July to September 2001). Annex to the Final report to the European Commission of PELASSES project (Contract 99/010) issued in 2002.

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

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

LIST of FIGURES:

Figure 5.1.5.3: Workshop Ageing of anchovy otoliths from the bay of Biscay: Modal Interpretation: 2 years old: A1. First winter ring, C15 Spawning check, A2, 2nd winter ring (at the edge). (Caught Mars 2000) (id.code: M2_15, length of 176 mm) (43% of agreement in the exchange programme and 71% in the workshop). Alternative interpretations over the right: 3 y.o.

Figure 5.1.5.4: Workshop Ageing of anchovy otoliths from the bay of Biscay: Modal Interpretation: 2 year old A1. First winter ring, C15 Spawning check. A2, 2nd winter ring (at the hyaline edge). (Caught Mars 2000, length 184 mm) (id.code: M2_19) (57% of agreement in the exchange programme and 100% in the workshop). Alternative interpretations given during the exchange programme can be seen over the right otolith for 3 or 1 year old).

Figure 5.1.5.5: Workshop Ageing of anchovy otoliths from the bay of Biscay: Modal Interpretation: 3 year old otolith: A1. First winter ring, C15 Spawning check. A2, 2nd winter ring (at the hyaline edge). (Caught April 2000, length 178 mm) (id.code: 00042724_11) (57% of agreement in the exchange and 86 % in the workshop). Alternative interpretation in the exchange programme 2 y.old.

Figure 5.1.5.6: Workshop Ageing of anchovy otoliths from the bay of Biscay: Modal Interpretation: 1 year old otolith: A1. First winter ring, C15 Spawning check. (Caught August 2000, length of 185 mmm) (id.code: M3_40) (43% of agreement in the exchange programme and 57 % in the workshop readings). Alternative interpretations over the right otolith for 2 year old, with A1 and A2 (2nd winter ring) and current year growth over the edge.

Figure 5.2.2.1: Anchovy otoliths from the Atlantics coast: Modal Interpretation: 1 year old otolith (typical example): A1. First winter ring, (Caught March 2001, length 134 mm) (id.code: AP34_16) (80% of agreement in the exchange programme).

Figure 5.2.2.2: Workshop ageing of otoliths from the Bay of Cadiz: Modal Interpretation: 2 year old otolith: A1. First winter ring, C15 Spawning check. A2, 2nd winter ring (at the hyaline edge). (Caught April 1999, length: 138 mm) (id.code: Golfo de Cádiz Abril99_11) (100% of agreement).

Figure 5.2.2.3: Alternative ageing of otoliths from the Bay of Cadiz : Modal Interpretation: 2 year old otolith: A1. First winter ring, C08 check before in its first year of life before first winter. A2, 2nd winter ring (at the hyaline edge). Alternative interpretations over the right 1 year old (Caught May 1999, Length 155 mm) (id.code: Golfo de Cádiz Mayo99_15) (60% of agreement).

Figure 5.2.2.4: Workshop ageing of otoliths from the Atlantics coast: Modal Interpretation: 3 years old: A1. First winter ring, A2, 2nd winter ring, A3 third winter ring at the hyaline edge. Exchange modal interpretation over the right 2 years old (Caught March 2001, length 176 mm) (id.code: AF31_92).

Figure 5.2.2.5: Workshop ageing of otoliths from the Atlantics coast: Modal Interpretation: 1 year old otolith, but difficult becouse A1 (First winter ring) was poorly marked (Caught March 2001, length of 138 mm) (id.code: AP34_26) (100% of agreement).

Figure 5.2.2.6: Workshop Ageing of otoliths from the bay of Cádiz: Modal Interpretation: 2 year old: A1. First winter ring (badly marked), C05 check before its first winter ring. A2, 2nd winter ring (at the hyaline edge) (100% of agreement). Alternative interpretations over the right 1 year old (Caught April 1999, 156 mm) (id.code: Golfo de Cádiz Abril99_1) (Considered to be age 1 in the exchange).

Figure 5.2.2.7: Ageing of otoliths from the Atlantics coast: Workshop Interpretation 2 years old but with very badly marked rings: A1 first winter ring, A2 at the hyaline edge. Exchange Modal interpretation 1 year old with. (Caught March 2001, length 176 mm) (id.code: AF31_91).

Figure 5.2.2.8: Workshop ageing of otoliths from the Bay of Cadiz : Modal Interpretation: 2 years old otolith: A1. First winter. A2, 2nd winter ring ring followed by an opaque growing band edge (60% of agreement). Alternative interpretation to the right: 1 year old (Caught June 1999, length 153 mm) (id.code: Golfo de Cádiz Junio99_31) (Considered 1y.o. in the exchange programme).

Figure 5.2.2.9: Ageing of otoliths from the Atlantics coast: Workshop Interpretation 3 year old otolith but A1(First winter ring) was too poorly marked, A2, 2nd winter ring and A3 3rd winter ring at the hyaline edge. Alternative interpretations in the exchange: 2 (Modal) or 1 year old (Caught March 2001, length 176 mm) (id.code: AF31_90).

Figure 5.2.3.1: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

Figure 5.2.3.2: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

Figure 5.2.3.3: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: The RELATIVE bias by MODAL age as estimated by all age readers combined.

Figure 5.2.3.4: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

Figure 5.2.4.1: Alternative ageing of otoliths from the Bay of Cadiz : Modal Interpretation: 1 year old otolith: A1. First winter ring, C07 check before in its first year of life before first winter. Alternative interpretations over the right 2 year old (Caught June 1999, length 120 mm) (id.code: Golfo de Cádiz Junio99_9) (80% of agreement in exchange and 100% in workshop).

Figure 5.2.4.2: Alternative ageing of otoliths from the Bay of Cadiz : Modal Interpretation: 2 year old otolith: A1. First winter ring, A2 second winter ring at the hyaline edge. Alternative interpretations over the right otolith: 1 year old (Caught April 1999, length 146 mm) (id.code: Golfo de Cádiz Abril99_12) (60% of agreement for the modal age in exchange and workshop).

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Table 5.1.4.1: ANCHOVY Workshop (Jan. 2002) SET A: BAY OF BISCAY: Upper table: The age compositions estimated by each age reader and all age readers combined. Midle table: The estimated mean length at age by age reader and by all age readers combined. Lower table: Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test.The inter-reader bias test and the reader against MODAL age bias test. And percentage of agreement between readers.

Table 5.1.4.4: ANCHOVY Workshop (Jan. 2002) SET A: BAY OF BISCAY: Otoliths read, CV's, percentage agreement and RELATIVE bias by month and by MODAL age.

Table 5.1.4.5: ANCHOVY Workshop (Jan. 2002) SET A: BAY OF BISCAY: Otoliths read, CV's, percentage agreement and RELATIVE bias by stratum and MODAL age.

Table 5.2.3.1: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: The set of otoliths and the age determinations performed during the workshop.

Table 5.2.3.2: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: The number of age readings, the coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

Table 5.2.3.3: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: Upper table: The age compositions estimated by each age reader and all age readers combined. Midle table: The estimated mean length at age by age reader and by all age readers combined. Lower table: Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test.The inter-reader bias test and the reader against MODAL age bias test. And percentage of agreement between readers.

Table 5.2.3.4: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: Otoliths read, CV's, percentage agreement and RELATIVE bias by month and by MODAL age.

Table 5.2.3.5: Workshop exercise (Jan. 2002) SET B: Bay of Cadiz: Otoliths read, CV's, percentage agreement and RELATIVE bias by stratum and MODAL age.

LIST OF ANNEXES

Annex 1- COMPLETE ADDRESSES OF ATTENDEES:

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Annex 2: Development of the Workshop:

Monday 14 January.
15:00 -16.00 Revision and adoption of the agenda and Organization of the workshop.
16:00-17:30 Presentation of ageing results from the otolith exchange programme concerning the Bay of Biscay and IXa anchovy otoliths: Comparisons of precision and accuracy against modal age. Evaluate current levels of ageing agreement (precision) among institutes.
17:30 –19:00 Identify major difficulties in age determination concerning observed disagreements for the Bay of Biscay.

Tuesday 15 January:
9:00 -11:00 (Cont…) Identify major difficulties in age determination concerning observed disagreements for the Bay of Biscay.
11:30- 13:30 Present and discuss AZTI's methodology for ageing anchovy otoliths from the Bay of Biscay.
15:30-16:30 Read the easy to read set of otoliths prepared by AZTI and data Inputs.
16:30-17:30 Presentation of ageing results and comparisons of precision and accuracy against actual age.
17:30 19:30: Evaluate improvements in ageing precision among institutes by the end of the meeting. Reading of the initial set A (Bay of Biscay) of Control Collections and data input.

Wednesday 16 January:
9:00 –11:00 Cont… "Reading of the two initial set A of Control Collections and data input.
11:30-12:30 Presentation of ageing results and comparisons of precision and accuracy against modal age. Evaluate current levels of ageing agreement (precision) among institutes.
12:30- 14:00 Open discussion and conclusions about:

- Establish an agenda of work for continuous tracing of quality in anchovy age determinations for the Bay of Biscay area
- Establish a digitalized agreed collection of otoliths for the Bay of Biscay anchovy.
- Expected Future research.

15:30 – 16:00 Definitive adoption of the agenda for Subarea IXa.
16:00-18:30 Identify major difficulties in age determination concerning observed disagreements in age determination from the exchange programme.

Thursday 17 January:
09:30-13:30 Identify major difficulties in age determination concerning observed disagreements. Discussion on the growth pattern of the otolith based upon agreements and disagreements on the exchange otoliths
15:30 - 17:30 Discuss on the possibilities to agreed on protocols for Ageing determination criteria of anchovy otoliths in Division IXa (and drafting of them)
17:30 - 19:30 Reading of part of the initial set B of Control Collections and data input, concerning the Bay of Cadiz (Division IXa south).

Friday 18 January:

09:00-10:30 Presentation of ageing results and comparisons of precision and accuracy against modal age. Evaluate current levels of ageing agreement (precision) among institutes.

10:30-14:00 Second discussion on the growth pattern and the otolith age reading in Division IXa and Basic ageing methodology. And discussion and conclusions about:

- Establish an agenda of work for continuous tracing of quality in anchovy age determinations for the division IXa
- Establish a digitalized agreed collection of otoliths for this division?
- Expected Future research.