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**Theme Session P**: Quality and Precision of Basic Data Underlying Fish Stock Assessment and Implications for Fisheries Management Advice

# Improvements in the quality of the basic horse mackerel age data during the last 20 years

by

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## Abstract

This paper describes the improvements in the quality of the basic horse mackerel age data within the ICES area over the last 20 years.

In 1983 growth parameters of horse mackerel estimated by different laboratories were compared and these indicated big differences in age reading. In 1984 a first ICES horse mackerel otolith exchange demonstrated a big difference of nearly a factor 2 in the age readings indicating that two very different ageing techniques were used for adult horse mackerel. A first workshop on horse mackerel age determination was held in 1987. However, the age reading problems could not be solved at this workshop without a proper validation of the ageing method. Finally in 1989 an agreement on the ageing method was reached when the ageing method was validated. A workshop in 1990 showed that the age readers applied quite well the agreed age reading method. In the second half of the 90's the comparison of the age reading results was considerably improved. Therefore, at an exchange in 1996 and at a workshop in 1999 it was possible to estimate the precision and the accuracy of the age readings of all individual readers and the whole group of age readers. The results of the workshop demonstrated an improvement in the precision but still a considerable underestimation of the older ages (bias) when reading broken/burnt otoliths of known age. This bias problem could not be solved since this seemed to be due to a bad visibility of the outer annual rings. Furthermore these workshop results enabled to estimate the effects of age reading errors on the horse mackerel assessment. An important conclusion of this analysis was that priority should be given to improving the accuracy (reducing underestimation of the older ages) over the improvement of the precision. This paper presents new results on a comparison of the otolith-processing techniques of both the broken/burnt and the stained sliced otoliths of known age. The results from the experienced age readers demonstrate that the processing technique of the stained sliced transverse sectioned otoliths can considerably reduce the bias in age reading and at the same time improve precision. However, some readers still need help to adapt to age reading otoliths from this new processing technique. Reading stained sliced otoliths is again a major step forward in the process of getting good quality basic horse mackerel age data. In future other staining techniques should be investigated to improve age reading results even more.

## Improvements in horse mackerel age reading during the period 1980 - 2000

In the beginning of the '80's the Netherlands started a commercial fishery on horse mackerel (*Trachurus trachurus* L.) in the English Channel and the Celtic Sea, because the quota for mackerel and herring were not sufficient anymore. At the Netherlands Institute for Fisheries Research the age reading of horse mackerel otoliths was started in 1982 without having any particular experience on age reading this species. First trials in age reading were carried out with broken/burnt otoliths.

At the meeting of the ICES Mackerel Working Group in 1983 a large range in horse mackerel growth parameters of the different countries was observed. This indicated that different age reading methods might be applied at the fisheries laboratories. Therefore an otolith exchange was recommended to detect these possible problems (ICES, 1984).

In 1984 a first ICES horse mackerel otolith exchange was carried out to check for possible inconsistencies in age reading (Eltink, 1985). A set of sliced transverse sectioned otoliths was compared with a set of whole and broken/burnt otoliths. Functional linear regressions of the age determinations were used for the analysis of the age readings of each pair of age readers. Both a slope of approximately 1 and an intercept of about 0 would indicate a similar age reading method by a pair of age readers. Standard deviations were estimated by otolith from the different age readings by the age readers and these were plotted against the estimated age per otolith together with the number of observations. In addition growth curves were plotted to indicate any differences in age reading techniques. Results showed that the Dutch and two Scottish age readers aged horse mackerel with a factor of about two higher than the other readers (the slopes of the regression lines differed approximately by a factor 2). The sectioned otoliths achieved lower standard deviations per age. It was concluded that consistent interpretation of horse mackerel otoliths was probably achievable up to 5 years old and that a more detailed study would be needed to improve the method of ageing of fish above 5 years of age. It was recommended to collect successive year's age compositions to test the age reading method for especially the older ages.

In Lowestoft, England an otolith workshop was organised in 1987 to solve this serious age reading problem (Anon., 1987). Again functional linear regressions of the age determinations were used for the analysis of the age readings of each pair of age readers. In addition the percentage frequency distributions of the standard deviations were plotted (standard deviations were estimated by otolith from the different age readings by the age readers). Results, however, showed the same phenomenon again, but now it was only the Dutch age reader who differed nearly a factor two compared to the rest of the age readers (Anon., 1987). This discrepancy was too large to get an agreement among the age readers at a workshop meeting.

Such a large discrepancy in age reading methods could only be solved by a validation study to indicate, which one would be correct. The indirect validation method of comparing successive year's age compositions was chosen for this purpose (Eltink and Kuiter, 1989). In principle only one of the two age reading methods can follow strong/weak year classes in successive year's age compositions, if the age reading methods differ about a factor two. Fortunately an extremely strong year class 1982 entered the fishery, which enabled such an indirect validation of the age reading method. In 1988 all age readers within the ICES area accepted the age reading method as applied by the Netherlands and they aged horse mackerel much older than they used to do. Horse mackerel can reach an age of approximately 40 years old and is therefore a long-lived species.

In 1988 and 1989 an exchange programme was carried out to evaluate how the readers were applying the agreed age reading technique (Borges, 1989). Functional linear regressions of the age determinations were used for the analysis of the age readings of each pair of age readers and again the percentage frequency distributions of the standard deviations were plotted. Furthermore the estimated mean length at age was plotted by age reader to help detecting any differences in the ageing method. The exchange sample from the southern area (Division IXa) caused difficulties showing much higher standard deviations compared to the western area. Not all age readers were included in the analysis of the otolith exchange. It was recommended to complete the exchange and to organise a workshop to solve the problems in age reading.

A second otolith workshop was held in 1990 at IPIMAR, Lisbon Portugal (ICES, 1991), at which all results of the exchange and the different otolith preparation methods should be evaluated and at which advise should be given on which age groups valid age readings can be achieved. The analysis of the age readings was the same as for the preceding exchange. A good improvement in the age readings from the exchange to the workshop was observed and the results indicated that a good agreement was achieved for all age groups. At the workshop a working document was presented on the comparative age readings of thin transverse sections of otoliths and broken/burnt otoliths (Vérin and Lorance, WD 1990). The authors indicated that there was a good agreement (59%) in the age readings from the two otolith-processing techniques with a slight tendency for higher age interpretations from the thin slices. Another working document was presented on thin transverse sections, which were burnt (Lucio and Arteche, WD 1990). This treatment gave a better contrast between the translucent and opaque zones. The workshop felt that similar ages might be read from the thin slices and recommended that research be carried out on the sectioning technique of obtaining thin slices of otoliths in order to improve the readability by increasing the contrast between the translucent and opaque zones. For the time being the broken/burnt otolith preparation technique was recommended.

In the 90’s no further investigations were carried out on the otolith-processing technique of sliced transverse sections, despite the workshop recommendation (ICES, 1991).

The ICES Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy recommended to organise a new horse mackerel otolith exchange in 1996, because new otolith readers would provide catch at age data for assessment purposes to the working group (ICES, 1996). The analysis of the 1996 otolith exchange differed considerably from the two earlier exchanges, because one otolith set contained otoliths of ‘known’ age and because the method of analysing the age determinations was substantially improved (Eltink, 1997). This enabled to provide information on the precision in age reading by age group (coefficient of variation) and on the accuracy in age reading by age group (bias) for all individual age readers and all readers combined. The results were presented as age bias plots in which the mean ages ± 2 standard deviations were plotted against the known ages and against the modal ages if the actual ages were not known. Relative bias is the age difference between mean estimated age and the modal age, while absolute bias is the age difference between mean estimated age and actual age. These age bias plots enabled a good visual comparison of both the precision and bias by the individual age readers and all age readers combined. The results from this exchange showed that a horse mackerel otolith workshop was needed to deal with serious problems in age reading. The ages of fish from approximately age 7 onwards were underestimated. This bias increased from age 8 gradually to approximately one year of underestimation at age 13. Furthermore the interpretation of the edge of otoliths appeared to be a major difficulty if these were taken from fish caught in the second half of the year, when both translucent and opaque edges occur (in the first half of the year only occur translucent edges).

To solve the problems in age reading a third horse mackerel otolith workshop was held at CEFAS, Lowestoft, England from 15-19 January 1999, at which 15 otolith readers participated (ICES, 1999). Three sets of otoliths were used, comprising only broken/burnt and whole otoliths of the extremely strong 1982 year class collected during the period 1983 -1995. These otoliths had a very high probability that the originally estimated age was correct and were therefore treated as otoliths of 'known' or 'actual' age. Two otolith sets were used for training. The first set contained otoliths with only translucent edges and the second otoliths with both translucent and opaque edges. The age reading comparisons of the first set showed that the precision was low for many readers and that the ages of the older fish were underestimated. The results from the second set showed that the underestimation of older ages was somewhat less. However, the precision was much lower compared to the first set, most likely due to difficulties in the interpretation of the otolith edge. At the end of the workshop a third set was used for estimating the improvement in age reading at the end of the workshop. The discussions on the results of both training sets and the discussions on specific difficult otoliths projected on a large screen resulted in an improvement of the ageing method of almost all readers. At this workshop there was an evident improvement in the precision and the accuracy for almost all readers, however, the underestimation of the older ages (bias) could not be reduced significantly. This underestimation of the older ages appears to be due to a bad visibility of the outer annual rings in the broken/burnt otoliths of the older fish. Therefore a recommendation was made to conduct a comparison between the sliced transverse section technique versus the traditional broken/burnt transverse section technique (ICES, 1999). New results on this comparison are available now and are presented in this paper in the section "Recent investigations in 2000 and 2001".

The estimation of the effect of age reading errors on the horse mackerel assessment was one of the terms of reference of the 1999 workshop. However, this term of reference could not be addressed at the workshop meeting itself, because the final results on precision, accuracy and absolute bias became only available at the end of the workshop from the last age reading exercise. Therefore, this analysis was carried out immediately after the workshop. This exercise was added as an addendum to the workshop report (see addendum of ICES, 1999). For the purpose of estimating the effect of age reading errors on the horse mackerel assessment an artificial true population was constructed over a 40-year period and true catches at age by year were calculated by applying certain fishing mortalities at age. The effect of age reading errors on the assessment was investigated by applying different levels of precision errors (CV of 5%, 10% and 15%) and by applying an absolute bias in age reading as observed at the workshop (from age 7 gradually increasing to a 1-year difference at age 13). The factor between largest and smallest recruitment also affects the level of errors in the assessment. Therefore 3 different factors were used to determine the errors in the assessment (factor 1, which implies constant recruitment and the factors 4 and 16). The assessments were tuned to absolute spawning stock biomass values as is done for the western horse mackerel with spawning stock biomass values obtained from egg surveys (ICES, 2001). The effects on the assessment caused by the age reading errors were expressed in percentage over- or underestimation of recruitment, fishing mortality, spawning stock biomass, population at age and selection pattern. This analysis indicated the effects caused by the age reading errors, but not by errors in biological sampling, proportion mature at age, fecundity and egg sampling for the biomass estimate. Age reading errors as were observed at the end of the 1999 workshop (CV=15% with bias) had the following estimated effects on the assessment of horse mackerel:

- Below average recruitment might be overestimated by 200% and above average recruitment underestimated by up to 35%. In addition the recruitment of the most recent years will be overestimated (gradually increasing up to 20% in the last year). The assessment will provide only very smoothed recruitment estimates and the difference between highest and lowest observed recruitment might be 5 times higher.
- Fishing mortality (F) will be 1-9% overestimated except in the last two or three years, when it might be slightly underestimated.
- Spawning stock biomass (SSB) will be 0-7% underestimated except in the last two or three years, when it might be slightly overestimated.
- The population at age in the last year will show the highest overestimation in the younger age groups, which gradually decreases up to age 10, becomes an underestimation from age 11 onwards and the 15+ age group shows a sudden increase in the underestimation. There is an additional effect of overestimating weak year classes and underestimating strong year classes.

- The fitted selection pattern in the last year will become dome shaped because of the bias. The highest selection is obtained at approximately age 7 or 8 (approximately 20-25% overestimation).

The effects of the observed age reading errors on the assessment of horse mackerel did not explain the problems in the stock assessment. Factors such as the assumption of natural mortality, proportion mature at age, spawning stock biomass estimates from egg surveys, age sampling by area/period, raising of age compositions to international catches, etc. are likely to have a larger impact on the assessment.

Figures 1–3 (from ICES, 1999) show respectively the effect of the age reading errors on the assessment given in percentage over- and underestimation of recruitment, of F and SSB over a 40-year period for recruitment factor 16 (difference between highest en lowest recruitment). Precision and precision combined with bias result in opposite effects on the estimation of recruitment, SSB and F. In many cases the options of CV's without bias provided worse recruitment estimates compared to CV's with bias. The effect of bias is highest when the precision is high (CV=5%). A low precision (CV=15%) combined with bias results in better assessment estimates than high precision (CV=5%) combined with bias. Figures 1-3 show that the effect of bias on the assessment is best compensated by a low precision (high CV of 15%). The opposite effects of both types of age reading errors cause this compensation. The precision errors cause an overestimation of age, because younger year classes are more abundant causing relatively more fish to be transferred from younger year classes to older ones than vice versa. Underestimation of the older ages (bias) has the opposite effect, because it causes relatively old fish to be transferred to younger year classes. It should be noted that if bias would be an overestimation of the older ages than precision and bias do not have an opposite effect, but would even increase each others effect.

From above it is evident that as first priority the bias in age reading should be reduced and only when this is achieved the precision should be improved (CV lower). Improving the precision considerably without reducing the bias would result in an even worse assessment, because precision errors have a compensating effect on the bias error. This clearly stresses the need of new investigations in order to reduce the bias in age reading of the older age groups (see next section).

## Recent investigations in 2000 and 2001

Introduction

At the horse mackerel otolith workshop in 1999 the bias problem of underestimating the ages of the older age groups could not be reduced (ICES, 1999). Even extensive discussions at the workshop did not help to reduce the bias problem. This underestimation of the older ages seems to be due to a bad visibility of the outer annual rings in the broken/burnt otoliths of the older fish. Furthermore the analysis on the effect of age reading errors on the stock assessment demonstrated that the precision in age reading should not be improved as long as the bias problem remained (addendum of ICES, 1999). This was because precision and bias (underestimation of age) are age reading errors, which have an opposite effect on the estimates of fishing mortality, spawning stock size and recruitment. It is a first priority to find otolith-processing techniques, which would improve the visibility of the outer annual rings and which would therefore reduce this bias problem. It would be a beneficial side effect if the precision would improve at the same time by this improved otolith-processing technique. This evaluation of the otolith-processing technique of sliced transverse sections technique is conducted following the 1999 workshop recommendation (ICES, 1999).

Material and Methods

The otolith sets K and L were used, which contained only otoliths of the extremely strong 1982 year class collected during the period 1983 -1995 and were treated as otoliths of 'known' or 'actual' age.

Otolith set K was used at the 1999 workshop for the estimation of the precision, accuracy and absolute bias as achieved at the end of the workshop. The whole otoliths of this set K were sliced to transverse sections in Ireland according to the workshop recommendation, while the other otoliths of each pair of otoliths were already broken/burnt according to the traditional method. At the Irish Fisheries Research Centre a number of different stains were tested to achieve the best visibility of the outer annual rings. Based on these results the sliced transverse sections of set K were stained with a light wood stain “Honeydue” (Sadolin). This stain was used neat from the tin and a very small amount was applied with a fine water colour paint brush to the surface. The following four best readers of the 1999 workshop read the otoliths:

| | | |
|---|---|---|
| Reader 1 | Mike Kerstan | South Africa |
| Reader 2 | Pablo Abaunza | Spain |
| Reader 3 | Eugene Mullins | Ireland |
| Reader 4 | Helga Gill | Norway |

Age reading of the broken/burnt otoliths of set K was not done, because the readings of these age readers were directly available from the workshop report (ICES, 1999). This set contained 153 pairs of otoliths.

Furthermore an otolith set L was compiled in order to estimate the effect of staining the transverse sliced sections with Neutral Red in comparison to the broken/burnt otolith preparation technique. Otolith set L contained both broken/burnt and whole otoliths. At the Netherlands Institute for Fisheries Research in IJmuiden, Netherlands transverse sections were cut from the whole otoliths. Only the best reader of the sliced transverse sectioned otoliths of set K (Pablo Abaunza, Spain) carried out the age reading for set L. Age reading took place both before and after staining the transverse sections with Neutral Red to estimate the effect of the staining itself. This age reader viewed both the broken/burnt and sliced transverse sections with reflected light. This set L contained 134 pairs of otoliths for comparison.

Results

**Table 1** contains the sample information of otolith set K and the input data of the age reading results from the four participating age readers by otolith-processing technique. The age reading results from the broken/burnt otoliths were taken from the workshop (ICES, 1999). The age reading results from the stained sliced transverse sections were obtained by exchanging these otoliths between the age readers. The age readings were compared to actual age. The percent agreement and the coefficient of variation were estimated for each pair of otoliths for both otolith-processing techniques combined.

**Table 2** shows by otolith-processing technique the number of age readings, the coefficient of variation (CV), the percent agreement and the absolute bias by actual age for each age reader and all age readers combined. For each otolith-processing technique a weighted mean CV and a weighted mean percent agreement are given by actual age, by reader and for all ages and readers combined. The precision in age reading is indicated by the coefficient of variation (CV). The weighted mean CV’s over all age groups combined indicate the precision in age reading by reader and for all readers combined. The best precision (low CV) is obtained for the broken/burnt method (7.4%) compared to the sliced transverse section method (12.8%). However, not all readers have a better precision for the broken/burnt method. The two first readers increased the precision by reading the sliced transverse sections (reduced CV by 0.8% and 1.0%), while the last two readers achieved a much worse precision (increased CV by 4.3% and 5.0%). The best percentage agreement is obtained for the broken/burnt method (60.1%) compared to the sliced transverse section method (54.0%). However, not all readers have a better percentage agreement for the broken/burnt method. The second reader increased the percentage agreement by reading the sliced transverse sections (increased considerably by 18.3% from 52.9% to 71.2%), while the other readers achieved a much worse percentage agreement (reduced percentage agreement by respectively 18.3%, 17.6% and 6.6%). The absolute bias by actual age for each individual age reader and all readers combined indicates the accuracy in age reading. These absolute bias estimates are plotted in Figure 4 and 5A/B and are explained below.

**Figure 4** shows the age bias plots in which the mean age recorded ±2stdev is plotted against the actual age. The age readings are in agreement with actual age when the mean age recorded is on the 1:1 equilibrium line (mean age recorded equal to actual age). Readers have an absolute bias in age reading when the mean age recorded is lower (underestimation of age) or higher (overestimation of age) than the actual age. The ±2stdev bars (no or small bars indicate high precision, while large bars indicate low precision) indicate the precision errors.

The following can be observed in Figure 4, when the broken/burnt and the sliced transverse section otolith-processing techniques are compared:

- Reader 1 increases precision, but overestimates ages over the whole age range;
- Reader 2 increases precision and reduces considerably the underestimation of the older age groups;
- Reader 3 and 4 reduce precision and increase the underestimation of the older age groups.

**Figures 5A** and **5B** show respectively by reader the precision (CV) in age reading by actual age for both otolith-processing techniques based on the age readings from set K (data from Table 2). Reader 1 and 2 improved the precision (reduced CV to just above 5%) especially over the age range 7-13 when age reading the sliced transverse sectioned otoliths and did not achieve such a high precision when reading the broken/burnt otoliths over the same age range. Reader 3 and 4 were not able to achieve a higher precision when age reading the older age groups of the sliced otoliths.

**Figures 6A** and **6B** show respectively by reader the absolute bias in age reading for both otolith-processing techniques based on the age readings from set K (data from Table 2). Reader 1 performed best in age reading the broken/burnt otoliths (Figure 6A), while reader 2 performed best in age reading the stained sliced transverse sections (Figure 6B). Reader 3 and 4 were not able to achieve a lower absolute bias when age reading the older age groups of the sliced otoliths.

**Table 3** contains the sample information of otolith set L and the input data of the age reading results from age reader 2 by otolith-processing technique. The age reading results of both unstained and Neutral Red stained sliced transverse sections are presented for estimating the effect of staining. The age readings were compared to actual age. The percent agreement and the coefficient of variation were estimated for each pair of otoliths for all otolith-processing techniques combined.

**Table 4** shows by otolith-processing technique the number of age readings, the coefficient of variation (CV), the percent agreement and the absolute bias by actual age for age reader 2 only. For each otolith-processing technique a weighted mean CV and a weighted mean percent agreement are given by actual age and for all ages combined for reader 2 only. The precision in age reading is indicated by the coefficient of variation (CV). The weighted mean CV's over all age groups combined indicate the precision in age reading for each otolith-processing technique by the same otolith reader. The best precision (low CV) is obtained for the broken/burnt method (9.9%) compared to the unstained and stained sliced transverse section method (CV's respectively 15.7% and 12.1%). Staining the sliced transverse sections with Neutral Red improved the precision in age reading (reduced CV by 3.6%) when compared to the unstained sliced transverse sections, but reduced the precision (increased CV by 2.2%) when compared to the broken/burnt otolith-processing technique. A slightly better percentage agreement is obtained for the broken/burnt method (60.3%) compared to the unstained and stained sliced transverse section method (respectively 57.6% and 56.0%). The absolute bias by actual age indicates the accuracy in age reading for the different otolith-processing techniques. These absolute bias estimates are plotted in Figure 7 and 8 and are explained below.

**Figure 7** shows for age reader 2 by otolith-processing technique the age bias plots in which the mean age recorded ±2stdev is plotted against the actual age. The small error bars concerning the age readings of the broken/burnt sections indicate that precision is best (CV lowest) for this otolith-processing technique. Furthermore the age underestimation is lowest for this technique. But the staining of sliced transverse sections with Neutral Red does not appear to result in a better precision and accuracy compared to broken/burnt section.

**Figure 8** shows for set L the precision (CV) in age reading by actual age and by otolith-processing technique based on the age readings from reader 2 (data from Table 4). Reader 2 did not improve the precision over the age range 7-13 when age reading the unstained and stained sliced transverse sectioned otoliths and performed best when age reading the broken/burnt otoliths.

**Figure 9** shows for set L the absolute bias in age reading by actual age and by otolith-processing technique based on the age readings from reader 2 (data from Table 4). Reader 2 did not reduce the absolute bias over the age range 7-13 when age reading the unstained and stained sliced transverse sectioned otoliths and performed best when age reading the broken/burnt otoliths.

In the text table below the reader's age against actual age bias test (non-parametrically with a one-sample Wilcoxon rank sum test) is presented:

| **SET K** | | Otolith-processing technique | |
|---|---|---|---|
| | | Broken/burnt | Sliced transv. |
| Reader 1 | MK S-Africa | * | * * |
| Reader 2 | PA Spain | * * | — |
| Reader 3 | EM Ireland | — | * * |
| Reader 4 | HG Norway | * * | * * |

| |
|---|
| — = no signs of bias ($p>0.05$) |
| * = possibility of bias ($0.01<p<0.05$) |
| * * = certainty of bias ($p<0.01$) |

| **SET L** | | Otolith-processing technique | | |
|---|---|---|---|---|
| | | Broken/burnt | Unstained sliced transv. | Stained sliced transv. |
| Reader 2 | PA Spain | * | — | — |

The Wilcoxon rank sum tests above show that the age readings of age reader 2 of the sliced transverse sections of both set K and L do not show signs of bias when these are compared to actual or known ages. But reader 2 appears to have a possibility or certainty of bias when age reading the broken/burnt otoliths.

## Discussion

The main aim of this study is to find an otolith-processing technique that reduces the underestimation of the older age groups. In this case the age readings of the traditional broken/burnt otoliths are to be compared to the age readings of the stained sliced transverse sectioned otoliths. For the evaluation of these two techniques one otolith of each fish has to be processed according the traditional broken/burnt technique while the other otolith has to be prepared according the sliced transverse section technique. The analysis of the age readings enables the estimation of the precision and the relative bias (accuracy) by age group for each processing technique, when otoliths of unknown age are used. However, if otoliths of known age are used it even enables the estimation of precision and absolute bias. Absolute bias is the difference between mean estimated age and the actual age. The estimation of the absolute bias is indispensable in this kind of otolith-processing technique evaluation. The otolith sets K and L contained only otoliths of the extremely strong 1982 year class, which were collected during the period 1983 -1995. These otoliths had a very high probability that the originally estimated age was correct and were therefore treated in the analysis as otoliths of 'known' or 'actual' age (ICES, 1999).

EFAN (European Fish Ageing Network (www.efan.no)) recommends the use of the guidelines and tools for age reading comparisons as described in Eltink *et al*. (2000). These guidelines and the program for age reading comparisons are an attempt to formalise and advertise the best features currently available in Europe. These guidelines concentrate on reference collections, on exchange schemes and age reading workshops, on the analysis of the age readings, on the digital imaging tools and the definition of terms. The evaluation of the two otolith-processing techniques in this study is in agreement with these guidelines.

Improving the precision considerably without reducing the bias would result in an even worse assessment, because precision errors have a compensating effect on the bias error (ICES, 1999). First priority should therefore be given to the reduction of the underestimation of the older ages (bias). A reduction of both bias and CV at the same time would of course be very welcome.

The underestimation of the older ages is probably caused by a bad visibility of the outer translucent and opaque rings. Vice versa, if the absolute bias and the CV in age reading of the older ages are estimated to have reduced, it is expected that visibility of the outer translucent and opaque zones has improved and that a better otolith-processing technique has been used.

It should be taken into account that the four age readers of otolith set K only did have experience in age reading the broken/burnt otoliths, but not in age reading the stained sliced transverse sections. Nevertheless reader 1 and 2 were able to improve the precision in age reading the sliced sections (reduced CV respectively from 8.0% to 7.2% and from 8.5% to 7.5%). Reader 2 could clearly reduce the underestimation of the older ages (7-13), but unfortunately reader 1 introduced a bias (overestimation of age), which is evident from ages 1-4 and from ages 7-12. Visibility of the outer annual rings has probably increased, because reader 1 and 2 improved the precision considerably over the age range 7-13 (Figure 5A and 5B) and reader 2 reduced the absolute bias (Figure 6A and 6B) over the age range 7-13. It is assumed that age reader 2 would not have been able to improve the precision and accuracy in age reading that much if the visibility of the outer rings would not have been improved by the stained sliced otoliths. Reader 2 increased the percentage agreement from 52% to 71%, because both the precision and accuracy improved. Reader 1 reduced the percentage agreement from 74% to 56%, because the effect from the introduced bias (accuracy error) was greater than the effect of the improved precision. Reader 3 and 4 appeared to have even more difficulties in adapting to the new otolith-processing technique, because they achieved the best precision and accuracy for the traditional broken/burnt otolith-processing technique. Readers 1, 3 and 4 probably need training from a qualified age reader on how to interpret the ring structures of the stained sliced transverse sections before they are able to achieve good age readings.

The bad age reading results from the “honeydue” stained sliced transverse sectioned otoliths by most of the readers should not be taken as a proof that the broken/burnt otolith-processing method should be preferred, because this is likely to be due to the inexperience of age reading the sliced otoliths. The best otolith reader achieved for this otolith-processing technique a considerable reduction of the bias in the age readings of the older age groups and at the same time an improved precision in the age readings. It is concluded that the otolith-processing method of “honeydue” stained sliced transverse sectioned otoliths is expected to provide more precise and more accurate age reading results than the traditional broken/burnt otoliths. This is a major step forward in the process of getting good quality basic horse mackerel age data.

Richter and McDermott (1990) experimented with a variety of different histological stains and otoliths of different species. They compared broken/burnt otoliths with stained sliced transverse sectioned otoliths and recommended the use of Neutral Red for staining the otoliths. Therefore, set L was used to test, if the staining with Neutral Red would even more improve the visibility of the outer rings. Only reader 2 was requested to carry out the age readings of set L, because reader 2 made the following improvements in age reading the sliced transverse sectioned otoliths of set K:

1. Precision: CV reduced considerably for age groups 6 and older (Table 2 and Figure 5AB);
2. Accuracy: absolute bias reduced considerably for age group 6 and older (Table 2 and Figure 6AB) and the percentage agreement improved by 18.3% (Table 2);
3. Wilcoxon rank sum test: no signs of bias when reading the sliced transverse sectioned otoliths.

The analysis of the age reading results of the unstained sliced transverse sectioned otoliths compared to the broken/burnt otoliths indicates that the age readings of the unstained sliced transverse section processing technique does not achieve a higher precision and accuracy than the broken/burnt otolith processing technique. It appears that the staining attributes most for the achievement of a high precision and accuracy. The age reading results of the sliced transverse sections stained with Neutral Red are rather disappointing. The precision and accuracy in age reading is highest for the broken/burnt otolith-preparation technique despite the staining with Neutral Red.

An important result of the analysis of the age readings of set L is that the age readings of the unstained sliced transverse sectioned otoliths are less precise and less accurate than age readings of the broken/burnt otoliths. It demonstrates that it is actually the staining that has to improve the visibility of the translucent and opaque rings and it is not the slicing itself that improves the visibility. Unfortunately the precision and accuracy in age reading were not estimated before the "honeydue" staining set K.

Further investigations should be carried out to improve the staining technique of the sliced transverse sections of the horse mackerel otoliths in order to improve the contrast between the translucent and opaque zones of the outer rings.

## Conclusions

It is concluded that the otolith-processing technique of sliced transverse sectioned horse mackerel otoliths stained with "honeydue" (Sadolin) can provide more precise and more accurate age reading results than the otolith-processing technique of the traditional broken/burnt otoliths. The best otolith reader achieved for this otolith-processing technique a considerable reduction of the absolute bias in the age readings of the older age groups and at the same time an improved precision in the age readings. This is a major step forward in the process of getting good quality basic horse mackerel age data.

Not all age readers experienced in age reading the broken/burnt otoliths were able to improve the precision and accuracy in age reading when reading the "honeydue" stained sliced transverse sectioned otoliths. This is likely due to the inexperience in age reading otoliths from this otolith-processing technique. It is recommended that inexperienced age readers get training from a qualified age reader.

Age readings from the unstained sliced transverse sectioned otoliths appeared to be worse than from the traditional broken/burnt otoliths. It demonstrates that it is actually the staining that has to improve the visibility of the translucent and opaque rings and it is not the slicing itself that improves the visibility. It is recommended that further investigations be carried out to improve the staining technique of the sliced transverse sections of the horse mackerel otoliths in order to improve even more the contrast between the translucent and opaque zones of the outer rings.

Ackowledgement

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## References

Anon., 1987. Report of the Horse Mackerel (Scad) Age Determination Workshop (Fisheries Laboratory Lowestoft 9-14 April 1987). Contract between the Commission of the European Communities and the United Kingdom Ministry of Agriculture, Fisheries and Food.

Borges M.F., 1989. Results of the Horse Mackerel (*Trachurus trachurus* L.) Otolith Exchange Programme during 1988 - 1989. ICES CM 1989/H:13, 24 pp.

Eltink A., 1985. Results of the Horse Mackerel (*Trachurus trachurus* L.) Otolith Exchange Program. ICES C.M. 1985/H:40, 17 pp.

Eltink A., 1997. Horse Mackerel Otolith Exchange in 1996. ICES CM 1997/HH:24 30pp.

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

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

Eltink, A.T.G.W., A.W. Newton, C. Morgado, M.T.G. Santamaria and J. Modin, 2000. Guidelines and tools for age reading comparisons. (PDF document version 1.0 October 2000) Internet: http://www.efan.no

ICES, 1984. Report of the Mackerel Working Group. ICES CM 1984/Assess:1 61pp.

ICES, 1996. Report of the Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy. ICES CM 1996/Assess:7 165pp.

ICES, 1991. Report of the Horse Mackerel (Scad) Otolith Reading Workshop. Lisbon, 21-27 November 1990. ICES C.M. 1991/H:59, 59 pp.

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

ICES, 2001. Report of the Working Group on the Assessment of Mackerel, Horse Mackerel, Sardine and Anchovy. ICES CM 2000/Assess:06 456pp.

Lucio, P. and L. Arteche, WD 1990. Proceedings in preparation and reading of *Trachurus trachurus* otoliths. Working document presented at the Horse Mackerel Otolith Reading Workshop, Lisbon, 21-27 November 1990.

Richter, H. and J.G. McDermott, 1990. The staining of fish otoliths for age determination. J. Fish Biol. (1990) 36, 773-779.

Vérin, Y. and P. Lorance, WD 1990. First trials of thin slices method for reading the horse mackerel otoliths (*Trachurus trachurus* L.). Working document presented at the Horse Mackerel Otolith Reading Workshop, Lisbon, 21-27 November 1990.

## Table 1 Horse Mackerel Otolith SET K Comparison of otolith-processing techniques

Broken/burnt otoliths compared with sliced transverse sectioned otoliths stained with Sadolin "honeydue"

| | | | | Broken/burnt otoliths | | | | Sliced transverse sections | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Orig. nr | Exch nr | Fish length | Landing month | MK S-Afr Reader 1 | PA Spain Reader 2 | EM Irel. Reader 3 | HG Norw. Reader 4 | MK S-Afr Reader 1 | PA Spain Reader 2 | EM Irel. Reader 3 | HG Norw. Reader 4 | **ACTUAL AGE** | **Percent agreement** | **Precision CV** |
| 1 | 61 | 14.2 | 10 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | **1** | 88% | 31% |
| 2 | 62 | 14.9 | 9 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | **1** | 88% | 31% |
| 3 | 63 | 18.9 | 10 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | **1** | 88% | 31% |
| 4 | 64 | 19.4 | 10 | 1 | 3 | 3 | 2 | 4 | 3 | 3 | 2 | **3** | 50% | 35% |
| 5 | 65 | 19.8 | 10 | 2 | 3 | 3 | 2 | 4 | 3 | 3 | 2 | **3** | 50% | 26% |
| 6 | 66 | 20.3 | 10 | 3 | 3 | 3 | 2 | 4 | 4 | 3 | 2 | **3** | 50% | 25% |
| 7 | 67 | 20.4 | 10 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | **2** | 88% | 17% |
| 8 | 68 | 20.6 | 9 | 4 | 4 | 4 | 3 | 5 | 4 | 4 | 3 | **4** | 63% | 17% |
| 9 | 69 | 20.8 | 4 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | **3** | 88% | 11% |
| 10 | 70 | 20.9 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | **3** | 100% | 0% |
| 11 | 71 | 21.2 | 10 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 2 | **3** | 75% | 18% |
| 12 | 72 | 21.4 | 10 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | **2** | 63% | 22% |
| 13 | 73 | 21.7 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | **4** | 63% | 17% |
| 14 | 74 | 21.7 | 9 | 4 | 4 | 4 | 4 | 5 | 4 | 4 | 3 | **4** | 75% | 13% |
| 15 | 75 | 21.8 | 10 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | **2** | 75% | 21% |
| 16 | 76 | 22.0 | 10 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | **2** | 75% | 21% |
| 17 | 77 | 22.3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | **3** | 75% | 14% |
| 18 | 78 | 22.4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | **3** | 88% | 12% |
| 19 | 79 | 22.9 | 10 | 2 | 3 | 3 | 2 | 3 | 2 | 3 | 2 | **2** | 50% | 21% |
| 20 | 80 | 23.1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | **3** | 88% | 12% |
| 21 | 81 | 23.2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | **4** | 75% | 12% |
| 22 | 82 | 23.3 | 4 | 4 | 5 | 4 | 4 | 5 | 4 | 3 | 3 | **4** | 50% | 19% |
| 23 | 83 | 23.3 | 10 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | **4** | 75% | 12% |
| 24 | 84 | 23.4 | 9 | 4 | 4 | 4 | 3 | 4 | 4 | 3 | 3 | **4** | 63% | 14% |
| 25 | 85 | 23.5 | 4 | 4 | 4 | 5 | 4 | 4 | 5 | 4 | 3 | **4** | 63% | 16% |
| 26 | 86 | 23.7 | 2 | 4 | 4 | 4 | 4 | 5 | 4 | 3 | 3 | **4** | 63% | 17% |
| 27 | 87 | 23.8 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | **4** | 100% | 0% |
| 28 | 88 | 24.0 | 2 | 4 | 5 | 4 | 4 | 4 | 5 | 3 | 4 | **4** | 63% | 16% |
| 29 | 89 | 24.1 | 9 | 5 | 6 | 6 | 5 | 8 | 7 | 7 | 4 | **7** | 25% | 22% |
| 30 | 90 | 24.3 | 3 | 5 | 6 | 5 | 5 | 5 | 5 | 5 | 4 | **5** | 75% | 11% |
| 31 | 1 | 24.3 | 9 | 5 | 6 | 7 | 5 | 6 | 6 | 5 | 5 | **6** | 38% | 13% |
| 32 | 2 | 24.5 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 4 | **4** | 88% | 9% |
| 33 | 3 | 24.5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 3 | **4** | 75% | 13% |
| 34 | 4 | 24.6 | 9 | 6 | 6 | 7 | 6 | 7 | 7 | 7 | 5 | **7** | 50% | 12% |
| 35 | 5 | 24.6 | 10 | 9 | 9 | 8 | 8 | 9 | 9 | 7 | 7 | **9** | 50% | 11% |
| 36 | 6 | 24.7 | 5 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 4 | **4** | 75% | 11% |
| 37 | 7 | 24.8 | 9 | 7 | 6 | 7 | 6 | 7 | 7 | 8 | 7 | **7** | 63% | 9% |
| 38 | 8 | 24.8 | 11 | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 6 | **6** | 50% | 10% |
| 39 | 9 | 25.0 | 11 | 9 | 8 | 9 | 8 | 9 | 9 | 10 | 8 | **9** | 50% | 8% |
| 40 | 10 | 25.1 | 3 | 8 | 8 | 8 | 9 | 8 | 8 | 8 | 8 | **8** | 88% | 4% |
| 41 | 11 | 25.1 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 | **5** | 75% | 10% |
| 42 | 12 | 25.1 | 9 | 12 | 11 | 10 | 9 | 11 | 10 | 9 | 8 | **10** | 25% | 13% |
| 43 | 13 | 25.3 | 4 | 3 | 4 | 4 | 4 | 5 | 4 | 3 | 4 | **4** | 63% | 17% |
| 44 | 14 | 25.3 | 10 | 7 | 7 | 9 | 7 | 8 | 8 | 9 | 8 | **8** | 38% | 11% |
| 45 | 15 | 25.4 | 3 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | **5** | 88% | 7% |
| 46 | 16 | 25.4 | 10 | 4 | 4 | 4 | 4 | 5 | 4 | 4 | 4 | **4** | 88% | 9% |
| 47 | 17 | 25.4 | 9 | 4 | 4 | 5 | 4 | 5 | 4 | 5 | 5 | **5** | 50% | 12% |
| 48 | 18 | 25.5 | 3 | 7 | 8 | 7 | 7 | 7 | 7 | 6 | 7 | **7** | 75% | 8% |
| 49 | 19 | 25.6 | 9 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | **7** | 75% | 7% |
| 50 | 20 | 25.6 | 9 | 5 | 5 | 6 | 5 | 6 | 6 | 6 | 5 | **6** | 50% | 10% |
| 51 | 21 | 25.7 | 2 | 6 | 7 | 6 | 6 | 7 | 6 | 6 | 6 | **6** | 75% | 7% |
| 52 | 22 | 25.7 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 5 | **4** | 75% | 13% |
| 53 | 23 | 25.7 | 9 | 8 | 7 | 7 | 6 | 8 | 8 | 8 | 5 | **8** | 50% | 16% |
| 54 | 24 | 25.8 | 2 | 6 | 7 | 6 | 6 | 6 | 6 | 6 | 5 | **6** | 75% | 9% |
| 55 | 25 | 25.8 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | **5** | 88% | 7% |
| 56 | 26 | 25.8 | 11 | 6 | 6 | 6 | 5 | 6 | 6 | 6 | 5 | **6** | 75% | 8% |
| 57 | 27 | 25.9 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | **4** | 100% | 0% |
| 58 | 28 | 25.9 | 10 | 9 | 9 | 10 | 9 | 9 | 9 | 9 | 8 | **9** | 75% | 6% |
| 59 | 29 | 26.0 | 3 | 5 | 5 | 7 | 5 | 5 | 7 | 7 | 5 | **5** | 63% | 18% |
| 60 | 30 | 26.0 | 10 | 11 | 8 | 10 | 8 | 12 | 11 | 9 | 11 | **11** | 38% | 15% |
| 61 | 121 | 26.2 | 9 | 5 | 5 | 6 | 5 | 6 | 5 | 5 | 5 | **5** | 75% | 9% |
| 62 | 122 | 26.3 | 11 | 9 | 8 | 9 | 8 | 10 | 10 | 9 | 9 | **9** | 50% | 8% |
| 63 | 123 | 26.3 | 11 | 12 | 10 | 11 | 10 | 12 | 11 | 11 | 11 | **11** | 50% | 7% |
| 64 | 124 | 26.4 | 3 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | **5** | 88% | 7% |
| 65 | 125 | 26.4 | 5 | 4 | 5 | 5 | 5 | 4 | 5 | 3 | 5 | **4** | 25% | 17% |
| 66 | 126 | 26.4 | 9 | 9 | 9 | 9 | 9 | 10 | 9 | 9 | 10 | **10** | 25% | 5% |
| 67 | 127 | 26.5 | 10 | 11 | 10 | 11 | 8 | 11 | 11 | 10 | 10 | **11** | 50% | 10% |
| 68 | 128 | 26.6 | 10 | 10 | 9 | 9 | 9 | 11 | 10 | 10 | 9 | **10** | 38% | 8% |
| 69 | 129 | 26.6 | 10 | 12 | 11 | 11 | 10 | 13 | 12 | 10 | 11 | **12** | 25% | 9% |
| 70 | 130 | 26.7 | 2 | 6 | 6 | 6 | 5 | 7 | 6 | 5 | 6 | **6** | 63% | 11% |
| 71 | 131 | 26.7 | 2 | 6 | 6 | 8 | 6 | 6 | 6 | 5 | 6 | **6** | 75% | 14% |
| 72 | 132 | 26.7 | 3 | 8 | 8 | 8 | 8 | 9 | 10 | 8 | 9 | **9** | 25% | 9% |
| 73 | 133 | 26.8 | 2 | 7 | 8 | 8 | 8 | 10 | 10 | 8 | 9 | **9** | 13% | 13% |
| 74 | 134 | 26.8 | 10 | 10 | 10 | 10 | 9 | 11 | 10 | 10 | 10 | **10** | 75% | 5% |
| 75 | 135 | 26.8 | 9 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | **6** | 75% | 8% |
| 76 | 136 | 26.9 | 2 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | **10** | 88% | 4% |
| 77 | 137 | 26.9 | 2 | 6 | 7 | 6 | 6 | 6 | 7 | 5 | 7 | **6** | 50% | 11% |
| 78 | 138 | 26.9 | 2 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | **6** | 88% | 6% |
| 79 | 139 | 26.9 | 11 | 10 | 8 | 10 | 10 | 10 | 11 | 10 | 10 | **10** | 75% | 8% |
| 80 | 140 | 27.0 | 9 | 11 | 10 | 11 | 11 | 11 | 12 | 10 | 12 | **11** | 50% | 7% |
| 81 | 141 | 27.1 | 1 | 11 | 10 | 12 | 10 | 12 | 11 | 10 | 12 | **11** | 25% | 8% |
| 82 | 142 | 27.1 | 10 | 11 | 10 | 12 | 12 | 12 | 11 | 11 | 11 | **11** | 50% | 6% |
| 83 | 143 | 27.2 | 3 | 8 | 7 | 8 | 7 | 8 | 9 | 8 | 8 | **8** | 63% | 8% |
| 84 | 144 | 27.2 | 11 | 12 | 12 | 11 | 12 | 14 | 12 | 11 | 11 | **12** | 50% | 8% |
| 85 | 145 | 27.3 | 2 | 6 | 7 | 6 | 5 | 6 | 7 | 6 | 12 | **6** | 50% | 32% |
| 86 | 146 | 27.3 | 3 | 9 | 8 | 9 | 8 | 9 | 9 | 8 | 5 | **9** | 50% | 17% |
| 87 | 147 | 27.3 | 11 | 8 | 8 | 7 | 7 | 8 | 7 | 8 | 8 | **7** | 38% | 7% |
| 88 | 148 | 27.4 | 1 | 10 | 10 | 11 | 12 | 12 | 11 | 11 | 8 | **11** | 38% | 12% |
| 89 | 149 | 27.5 | 9 | 7 | 10 | 10 | - | - | 10 | 9 | 9 | **9** | 33% | 13% |
| 90 | 150 | 27.5 | 9 | 10 | 10 | 10 | 11 | 12 | 10 | 10 | 11 | **11** | 25% | 7% |

## Table 1 Continued

| | | | | Broken/burnt otoliths | | | | Sliced transverse sections | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Orig. nr | Exch nr | Fish length | Landing month | MK S-Afr Reader 1 | PA Spain Reader 2 | EM Irel. Reader 3 | HG Norw. Reader 4 | MK S-Afr Reader 1 | PA Spain Reader 2 | EM Irel. Reader 3 | HG Norw. Reader 4 | **ACTUAL AGE** | **Percent agreement** | **Precision CV** |
| 91 | 31 | 27.6 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 7 | **5** | 88% | 13% |
| 92 | 32 | 27.6 | 9 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 6 | **5** | 75% | 11% |
| 93 | 33 | 27.7 | 2 | 9 | 9 | 8 | 9 | - | 8 | 6 | 8 | **9** | 43% | 13% |
| 94 | 34 | 27.7 | 11 | 10 | 9 | 9 | 11 | 11 | 10 | 10 | 10 | **10** | 50% | 8% |
| 95 | 35 | 27.7 | 11 | 6 | 6 | 7 | 5 | 7 | 6 | 6 | 6 | **6** | 63% | 10% |
| 96 | 36 | 27.8 | 1 | 13 | 12 | 12 | 13 | 13 | 12 | 11 | 12 | **13** | 38% | 6% |
| 97 | 37 | 27.8 | 2 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | **8** | 88% | 4% |
| 98 | 38 | 27.8 | 11 | 8 | 8 | 8 | 8 | 9 | 8 | 8 | 8 | **8** | 88% | 4% |
| 99 | 39 | 27.8 | 9 | 11 | 11 | 11 | 9 | 12 | 11 | 10 | 11 | **12** | 13% | 8% |
| 100 | 40 | 27.9 | 11 | 6 | 6 | 7 | 6 | 6 | 6 | 6 | 6 | **6** | 88% | 6% |
| 101 | 41 | 28.0 | 2 | 9 | 9 | 10 | 9 | 12 | 12 | 10 | 11 | **10** | 25% | 13% |
| 102 | 42 | 28.1 | 2 | 6 | 6 | 6 | 5 | 6 | 6 | 7 | 6 | **6** | 75% | 9% |
| 103 | 43 | 28.1 | 11 | 12 | 12 | 12 | 12 | 13 | 11 | 11 | 12 | **12** | 63% | 5% |
| 104 | 44 | 28.2 | 1 | 12 | 12 | 12 | 12 | 13 | 12 | 11 | 12 | **13** | 13% | 4% |
| 105 | 45 | 28.2 | 9 | 12 | 9 | 11 | 11 | 13 | 13 | 11 | 10 | **12** | 13% | 12% |
| 106 | 46 | 28.4 | 1 | 12 | 12 | 12 | 12 | 12 | 12 | 11 | 12 | **13** | 0% | 3% |
| 107 | 47 | 28.5 | 3 | 8 | 8 | 7 | 8 | 8 | 8 | 7 | 8 | **8** | 75% | 6% |
| 108 | 48 | 28.7 | 2 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | **5** | 88% | 7% |
| 109 | 49 | 28.7 | 3 | 9 | 9 | 8 | 9 | 10 | 10 | 8 | 10 | **9** | 38% | 9% |
| 110 | 50 | 28.7 | 9 | 10 | 11 | 12 | 10 | 13 | 12 | 11 | 11 | **12** | 25% | 9% |
| 111 | 51 | 28.8 | 3 | 11 | 11 | 11 | 10 | 11 | 10 | 9 | 10 | **10** | 38% | 7% |
| 112 | 52 | 28.8 | 11 | 7 | 7 | 7 | 8 | 8 | 8 | 7 | 7 | **7** | 63% | 7% |
| 113 | 53 | 29.0 | 2 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | **9** | 100% | 0% |
| 114 | 54 | 29.1 | 1 | 13 | 12 | 13 | 13 | 13 | 13 | 11 | 13 | **13** | 75% | 6% |
| 115 | 55 | 29.1 | 10 | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 5 | **5** | 63% | 10% |
| 116 | 56 | 29.2 | 1 | 13 | 12 | 14 | 14 | - | 12 | 7 | 9 | **13** | 14% | 23% |
| 117 | 57 | 29.2 | 10 | 5 | 5 | 6 | 6 | 5 | 5 | 6 | 5 | **5** | 63% | 10% |
| 118 | 58 | 29.3 | 1 | 13 | 11 | 12 | 14 | 13 | 13 | 12 | 12 | **13** | 38% | 7% |
| 119 | 59 | 29.3 | 2 | 7 | 6 | 6 | 7 | 8 | 7 | 7 | 6 | **7** | 50% | 10% |
| 120 | 60 | 29.3 | 3 | 7 | 7 | 7 | 6 | 6 | 6 | 5 | 11 | **7** | 38% | 26% |
| 121 | 91 | 29.3 | 11 | 6 | 7 | 7 | 5 | - | 5 | 6 | 5 | **6** | 29% | 15% |
| 122 | 92 | 29.5 | 2 | 8 | 7 | 6 | 6 | 8 | 7 | 7 | 6 | **7** | 38% | 12% |
| 123 | 93 | 29.6 | 9 | 13 | 12 | 11 | 12 | 12 | 11 | 10 | 10 | **12** | 38% | 9% |
| 124 | 94 | 29.7 | 1 | 14 | 13 | 12 | 13 | 14 | 13 | 12 | 11 | **13** | 38% | 8% |
| 125 | 95 | 29.8 | 3 | 12 | 11 | 11 | 10 | 12 | 11 | 10 | 11 | **11** | 50% | 7% |
| 126 | 96 | 29.8 | 9 | 9 | 9 | 11 | 9 | 10 | 9 | 9 | 9 | **9** | 75% | 8% |
| 127 | 97 | 30.0 | 1 | 12 | 12 | 11 | 11 | 12 | 13 | 11 | 13 | **13** | 25% | 7% |
| 128 | 98 | 30.2 | 10 | 8 | 8 | 9 | 6 | 9 | 8 | 7 | 8 | **8** | 50% | 13% |
| 129 | 99 | 30.3 | 1 | 11 | 10 | 9 | 11 | 12 | 11 | 10 | 10 | **11** | 38% | 9% |
| 130 | 100 | 30.5 | 1 | 13 | 10 | 10 | 11 | 11 | 12 | 10 | 12 | **11** | 25% | 10% |
| 131 | 101 | 30.6 | 2 | 7 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | **7** | 63% | 7% |
| 132 | 102 | 30.8 | 1 | 10 | 11 | 12 | 10 | 11 | 12 | 9 | 8 | **12** | 25% | 14% |
| 133 | 103 | 31.0 | 3 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 | **8** | 75% | 6% |
| 134 | 104 | 31.1 | 1 | 11 | 11 | 12 | 7 | 13 | 12 | 11 | 12 | **12** | 38% | 16% |
| 135 | 105 | 31.3 | 2 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 7 | **7** | 88% | 5% |
| 136 | 106 | 31.3 | 11 | 6 | 6 | 7 | 5 | 5 | 6 | 6 | 7 | **6** | 50% | 13% |
| 137 | 107 | 31.7 | 1 | 13 | 12 | 13 | 13 | - | 12 | 8 | 10 | **13** | 43% | 16% |
| 138 | 108 | 31.8 | 2 | 10 | 10 | 11 | 11 | 10 | 10 | 9 | 11 | **10** | 50% | 7% |
| 139 | 109 | 32.2 | 1 | 13 | 11 | 12 | 12 | 12 | 12 | 9 | 13 | **12** | 50% | 11% |
| 140 | 110 | 32.2 | 2 | 8 | 8 | 10 | 11 | - | 8 | 6 | 7 | **8** | 43% | 21% |
| 141 | 111 | 32.2 | 2 | 7 | 7 | 7 | 7 | 7 | 7 | 6 | 11 | **7** | 75% | 20% |
| 142 | 112 | 32.3 | 1 | 12 | 11 | 11 | 12 | 12 | 12 | 8 | 10 | **12** | 50% | 13% |
| 143 | 113 | 32.4 | 2 | 9 | 9 | 8 | 10 | 10 | 11 | 9 | 11 | **10** | 25% | 11% |
| 144 | 114 | 32.5 | 1 | 11 | 12 | 10 | 11 | 11 | 12 | 10 | 9 | **11** | 38% | 10% |
| 145 | 115 | 32.6 | 2 | 10 | 10 | 9 | 10 | 10 | 10 | 8 | 13 | **10** | 63% | 14% |
| 146 | 116 | 32.9 | 1 | 11 | 11 | 11 | 13 | 12 | 12 | 11 | 11 | **12** | 25% | 7% |
| 147 | 117 | 33.0 | 1 | 14 | 12 | 12 | 12 | - | 13 | 8 | - | **13** | 17% | 17% |
| 148 | 118 | 33.4 | 1 | 13 | 12 | 12 | 13 | 12 | 13 | 11 | 13 | **13** | 50% | 6% |
| 149 | 119 | 33.7 | 1 | 12 | 11 | 11 | 12 | 12 | 12 | 11 | 12 | **12** | 63% | 4% |
| 150 | 120 | 34.0 | 1 | 12 | 12 | 11 | 12 | - | 11 | 10 | 10 | **12** | 43% | 8% |
| 151 | 151 | 34.4 | 1 | 11 | 11 | 11 | 13 | 11 | 11 | 12 | 11 | **11** | 75% | 7% |
| 152 | 152 | 34.7 | 10 | 8 | 10 | 12 | 12 | 9 | 9 | 10 | 7 | **8** | 13% | 18% |
| 153 | 153 | 35.1 | 1 | 11 | 11 | 12 | 12 | - | 11 | 9 | 12 | **12** | 43% | 10% |
| | | | Total read | 153 | 153 | 153 | 152 | 144 | 153 | 153 | 152 | | **56.8%** | **11.5%** |
| | | | Total NOT read | 0 | 0 | 0 | 1 | 9 | 0 | 0 | 1 | | | |

**Table 2** By otolith-processing technique the number of age readings, the coefficient of variation (CV), the percent agreement and the absolute bias are presented by actual age for each age reader and for all readers combined. For each otolith-processing technique a weighted mean CV and a weighted mean percent agreement are given by actual age, by reader and for all ages and readers combined.

## SET K Comparison of otolith-processing techniques

| Nr AGE READINGS | | Broken/burnt otoliths | | | | | Sliced transverse sections | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ACTUAL | MK S-Afr | PA Spain | EM Irel. | HG Norw. | | MK S-Afr | PA Spain | EM Irel. | HG Norw. | |
| | AGE | Reader 1 | Reader 2 | Reader 3 | Reader 4 | TOTAL | Reader 1 | Reader 2 | Reader 3 | Reader 4 | TOTAL |
| | 1 | 3 | 3 | 3 | 3 | 12 | 3 | 3 | 3 | 3 | 12 |
| | 2 | 5 | 5 | 5 | 5 | 20 | 5 | 5 | 5 | 5 | 20 |
| | 3 | 9 | 9 | 9 | 9 | 36 | 9 | 9 | 9 | 9 | 36 |
| | 4 | 19 | 19 | 19 | 19 | 76 | 19 | 19 | 19 | 19 | 76 |
| | 5 | 13 | 13 | 13 | 13 | 52 | 13 | 13 | 13 | 13 | 52 |
| | 6 | 17 | 17 | 17 | 17 | 68 | 16 | 17 | 17 | 17 | 67 |
| | 7 | 13 | 13 | 13 | 13 | 52 | 13 | 13 | 13 | 13 | 52 |
| | 8 | 11 | 11 | 11 | 11 | 44 | 10 | 11 | 11 | 11 | 43 |
| | 9 | 12 | 12 | 12 | 11 | 47 | 10 | 12 | 12 | 12 | 46 |
| | 10 | 12 | 12 | 12 | 12 | 48 | 12 | 12 | 12 | 12 | 48 |
| | 11 | 13 | 13 | 13 | 13 | 52 | 13 | 13 | 13 | 13 | 52 |
| | 12 | 15 | 15 | 15 | 15 | 60 | 13 | 15 | 15 | 15 | 58 |
| | 13 | 11 | 11 | 11 | 11 | 44 | 8 | 11 | 11 | 10 | 40 |
| Total | 1-13 | 153 | 153 | 153 | 152 | 611 | 144 | 153 | 153 | 152 | 602 |

| COEFF. of VAR. | | Broken/burnt otoliths | | | | | Sliced transverse sections | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ACTUAL | MK S-Afr | PA Spain | EM Irel. | HG Norw. | ALL | MK S-Afr | PA Spain | EM Irel. | HG Norw. | ALL |
| | AGE | Reader 1 | Reader 2 | Reader 3 | Reader 4 | Readers | Reader 1 | Reader 2 | Reader 3 | Reader 4 | Readers |
| | 1 | 0% | 0% | 0% | 0% | 0.0% | 0% | 0% | 0% | 0% | 40.0% |
| | 2 | 0% | 20% | 21% | 0% | 13.5% | 0% | 0% | 21% | 0% | 22.7% |
| | 3 | 27% | 0% | 0% | 19% | 9.3% | 14% | 14% | 16% | 21% | 19.9% |
| | 4 | 6% | 10% | 8% | 10% | 5.1% | 11% | 10% | 21% | 19% | 16.8% |
| | 5 | 6% | 8% | 12% | 10% | 6.1% | 5% | 16% | 17% | 15% | 11.7% |
| | 6 | 8% | 11% | 10% | 9% | 10.3% | 9% | 7% | 10% | 26% | 10.4% |
| | 7 | 12% | 11% | 8% | 13% | 7.2% | 9% | 6% | 12% | 29% | 13.5% |
| | 8 | 4% | 11% | 17% | 23% | 8.7% | 6% | 7% | 14% | 13% | 8.9% |
| | 9 | 9% | 8% | 11% | 6% | 6.7% | 5% | 7% | 13% | 16% | 9.8% |
| | 10 | 9% | 9% | 9% | 8% | 6.1% | 6% | 7% | 7% | 12% | 8.5% |
| | 11 | 7% | 9% | 8% | 14% | 9.3% | 4% | 5% | 7% | 11% | 8.5% |
| | 12 | 8% | 7% | 4% | 14% | 7.6% | 6% | 5% | 10% | 11% | 10.4% |
| | 13 | 5% | 4% | 6% | 7% | 4.9% | 6% | 4% | 17% | 11% | 12.5% |
| Weighted mean | 1-13 | 8.0% | 8.5% | 8.8% | 11.2% | 7.4% | 7.2% | 7.5% | 13.1% | 16.2% | 12.8% |
| | RANKING | 3 | 4 | 5 | 6 | | 1 | 2 | 7 | 8 | |

| AGREEMENT (%) | | Broken/burnt otoliths | | | | | Sliced transverse sections | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ACTUAL | MK S-Afr | PA Spain | EM Irel. | HG Norw. | | MK S-Afr | PA Spain | EM Irel. | HG Norw. | |
| | AGE | Reader 1 | Reader 2 | Reader 3 | Reader 4 | ALL | Reader 1 | Reader 2 | Reader 3 | Reader 4 | ALL |
| | 1 | 100% | 100% | 100% | 100% | 100% | 0% | 100% | 100% | 100% | 75% |
| | 2 | 100% | 80% | 40% | 100% | 80% | 0% | 100% | 40% | 100% | 60% |
| | 3 | 78% | 100% | 100% | 67% | 86% | 33% | 78% | 78% | 56% | 61% |
| | 4 | 95% | 79% | 89% | 84% | 87% | 68% | 79% | 32% | 37% | 54% |
| | 5 | 92% | 85% | 69% | 77% | 81% | 92% | 62% | 54% | 69% | 69% |
| | 6 | 76% | 53% | 65% | 41% | 59% | 69% | 82% | 65% | 53% | 67% |
| | 7 | 62% | 46% | 69% | 46% | 56% | 54% | 85% | 54% | 38% | 58% |
| | 8 | 91% | 64% | 45% | 36% | 59% | 70% | 73% | 45% | 64% | 63% |
| | 9 | 75% | 50% | 33% | 45% | 51% | 60% | 50% | 42% | 50% | 50% |
| | 10 | 58% | 33% | 42% | 42% | 44% | 50% | 67% | 42% | 50% | 52% |
| | 11 | 62% | 15% | 46% | 38% | 40% | 38% | 69% | 23% | 46% | 44% |
| | 12 | 47% | 27% | 40% | 53% | 42% | 46% | 60% | 0% | 27% | 33% |
| | 13 | 55% | 9% | 18% | 45% | 32% | 50% | 55% | 0% | 30% | 33% |
| Weighted mean | 1-13 | 73.9% | 52.9% | 57.5% | 55.9% | 60.1% | 55.6% | 71.2% | 39.9% | 49.3% | 54.0% |
| | RANKING | 1 | 6 | 3 | 4 | | 5 | 2 | 8 | 7 | |

| ABSOLUTE BIAS | | Broken/burnt otoliths | | | | | Sliced transverse sections | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ACTUAL | MK S-Afr | PA Spain | EM Irel. | HG Norw. | | MK S-Afr | PA Spain | EM Irel. | HG Norw. | |
| | AGE | Reader 1 | Reader 2 | Reader 3 | Reader 4 | ALL | Reader 1 | Reader 2 | Reader 3 | Reader 4 | ALL |
| | 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.25 |
| | 2 | 0.00 | 0.20 | 0.60 | 0.00 | 0.20 | 1.00 | 0.00 | 0.60 | 0.00 | 0.40 |
| | 3 | -0.33 | 0.00 | 0.00 | -0.33 | -0.17 | 0.67 | 0.22 | -0.22 | -0.44 | 0.06 |
| | 4 | -0.05 | 0.21 | 0.11 | -0.05 | 0.05 | 0.32 | 0.21 | -0.37 | -0.42 | -0.07 |
| | 5 | -0.08 | 0.00 | 0.38 | -0.08 | 0.06 | 0.08 | 0.15 | 0.08 | 0.08 | 0.10 |
| | 6 | -0.24 | 0.12 | 0.41 | -0.59 | -0.07 | 0.06 | 0.06 | -0.24 | 0.18 | 0.01 |
| | 7 | -0.15 | -0.08 | -0.15 | -0.31 | -0.17 | 0.31 | 0.00 | -0.23 | 0.08 | 0.04 |
| | 8 | -0.09 | -0.09 | 0.55 | 0.18 | 0.14 | 0.30 | 0.09 | -0.18 | -0.55 | -0.09 |
| | 9 | -0.42 | -0.33 | -0.08 | -0.55 | -0.34 | 0.40 | 0.33 | -0.67 | -0.67 | -0.17 |
| | 10 | 0.00 | -0.42 | -0.33 | -0.25 | -0.25 | 0.58 | 0.25 | -0.67 | 0.25 | 0.10 |
| | 11 | 0.15 | -0.85 | -0.31 | -0.38 | -0.35 | 0.62 | 0.15 | -0.69 | -0.31 | -0.06 |
| | 12 | -0.40 | -0.87 | -0.60 | -0.93 | -0.70 | 0.46 | -0.27 | -1.87 | -1.07 | -0.72 |
| | 13 | -0.09 | -1.00 | -0.73 | -0.27 | -0.52 | -0.25 | -0.45 | -2.73 | -1.30 | -1.25 |
| Weighted mean | 1-13 | -0.14 | -0.25 | -0.03 | -0.32 | -0.19 | 0.36 | 0.07 | -0.63 | -0.34 | -0.14 |
| | RANKING | 3 | 4 | 1 | 5 | | 7 | 2 | 8 | 6 | |

| Overall ranking | Broken/burnt otoliths | | | | Sliced transverse sections | | | |
|---|---|---|---|---|---|---|---|---|
| | MK S-Afr | PA Spain | EM Irel. | HG Norw. | MK S-Afr | PA Spain | EM Irel. | HG Norw. |
| | Reader 1 | Reader 2 | Reader 3 | Reader 4 | Reader 1 | Reader 2 | Reader 3 | Reader 4 |
| king Coefficient of Variation | 3 | 4 | 5 | 6 | 1 | 2 | 7 | 8 |
| king Percentage Agreement | 1 | 6 | 3 | 4 | 5 | 2 | 8 | 7 |
| Ranking Absolute bias | 3 | 4 | 1 | 5 | 7 | 2 | 8 | 6 |
| OVERALL RANKING | 2 | 5 | 3 | 6 | 4 | 1 | 8 | 7 |

## Table 3 Horse Mackerel Otolith SET L Comparison of otolith-processing techniques

Broken/burnt otoliths compared with unstained and stained (Neutral Red) sliced transverse sectioned otoliths

| | | | | | | Broken/burnt otoliths | nstained sliced section | Stained sliced sections | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample year | Sample no | Fish no | Fish length | Otol. no | Landing month | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 | ACTUAL age | Percent agreemen | Precision CV |
| 83 | 20 | 26 | 14 | 1 | 10 | 1 | 1 | 1 | **1** | 100% | 0% |
| 83 | 21 | 6 | 19 | 2 | 10 | 2 | 2 | 1 | **1** | 33% | 35% |
| 85 | 55 | 6 | 19 | 3 | 10 | 2 | 3 | 3 | **3** | 67% | 22% |
| 85 | 42 | 40 | 20 | 4 | 10 | 3 | 3 | 3 | **3** | 100% | 0% |
| 85 | 50 | 30 | 20 | 5 | 10 | 4 | 3 | 3 | **3** | 67% | 17% |
| 85 | 50 | 39 | 21 | 8 | 10 | 3 | 3 | 3 | **3** | 100% | 0% |
| 85 | 3 | 30 | 21 | 9 | 3 | 3 | 3 | 3 | **3** | 100% | 0% |
| 85 | 55 | 23 | 21 | 10 | 10 | 4 | 3 | 3 | **3** | 67% | 17% |
| 86 | 22 | 12 | 21 | 11 | 9 | 3 | 4 | 4 | **4** | 67% | 16% |
| 85 | 5 | 26 | 22 | 12 | 4 | 4 | 2 | 3 | **3** | 33% | 33% |
| 84 | 27 | 4 | 22 | 13 | 10 | 3 | 2 | 2 | **2** | 67% | 25% |
| 84 | 30 | 36 | 22 | 15 | 10 | 4 | 3 | 2 | **2** | 33% | 33% |
| 85 | 55 | 25 | 22 | 16 | 10 | 3 | 3 | 3 | **3** | 100% | 0% |
| 85 | 5 | 28 | 22 | 17 | 4 | 4 | 3 | 4 | **3** | 33% | 16% |
| 86 | 11 | 2 | 23 | 18 | 4 | 4 | 5 | 4 | **4** | 67% | 13% |
| 86 | 32 | 2 | 23 | 19 | 10 | 3 | 4 | 3 | **4** | 33% | 17% |
| 84 | 30 | 47 | 23 | 20 | 10 | 3 | 2 | 3 | **2** | 33% | 22% |
| 85 | 3 | 47 | 23 | 21 | 3 | 3 | 3 | 3 | **3** | 100% | 0% |
| 85 | 5 | 31 | 23 | 22 | 4 | 3 | 3 | 3 | **3** | 100% | 0% |
| 84 | 27 | 23 | 23 | 23 | 10 | 3 | 2 | 2 | **2** | 67% | 25% |
| 86 | 14 | 3 | 23 | 24 | 5 | 4 | 4 | 3 | **4** | 67% | 16% |
| 84 | 28 | 33 | 24 | 26 | 10 | 3 | 3 | 3 | **2** | 0% | 0% |
| 86 | 10 | 4 | 24 | 27 | 4 | 4 | 4 | 4 | **4** | 100% | 0% |
| 86 | 14 | 12 | 24 | 28 | 5 | 4 | 4 | 4 | **4** | 100% | 0% |
| 86 | 11 | 11 | 24 | 29 | 4 | - | 4 | 4 | **4** | 100% | 0% |
| 86 | 39 | 7 | 24 | 30 | 10 | 4 | 4 | 4 | **4** | 100% | 0% |
| 86 | 11 | 9 | 24 | 31 | 4 | 4 | 5 | 4 | **4** | 67% | 13% |
| 87 | 9 | 3 | 25 | 32 | 3 | 5 | 4 | 5 | **5** | 67% | 12% |
| 90 | 55 | 10 | 25 | 33 | 10 | 8 | 3 | 7 | **8** | 33% | 44% |
| 88 | 1 | 3 | 25 | 34 | 2 | 6 | 4 | 6 | **6** | 67% | 22% |
| 91 | 63 | 12 | 25 | 35 | 10 | 8 | 3 | 8 | **9** | 0% | 46% |
| 86 | 37 | 2 | 25 | 36 | 11 | 4 | 3 | 3 | **4** | 33% | 17% |
| 87 | 6 | 5 | 25 | 37 | 3 | 5 | 3 | 5 | **5** | 67% | 27% |
| 88 | 33 | 6 | 25 | 38 | 9 | 6 | 5 | 6 | **6** | 67% | 10% |
| 90 | 46 | 2 | 25 | 39 | 9 | 8 | 5 | 8 | **8** | 67% | 25% |
| 86 | 5 | 3 | 25 | 40 | 3 | - | 3 | 4 | **4** | 50% | 20% |
| 89 | 48 | 17 | 25 | 41 | 9 | 7 | 7 | 6 | **7** | 67% | 9% |
| 86 | 1 | 13 | 25 | 42 | 2 | 5 | 4 | 4 | **4** | 67% | 13% |
| 86 | 11 | 14 | 25 | 43 | 4 | 4 | 4 | 5 | **4** | 67% | 13% |
| 86 | 32 | 18 | 25 | 44 | 10 | 5 | 4 | 5 | **4** | 33% | 12% |
| 92 | 57 | 5 | 25 | 45 | 11 | 10 | 11 | 15 | **10** | 33% | 22% |
| 87 | 6 | 9 | 25 | 46 | 3 | 5 | 5 | 7 | **5** | 67% | 20% |
| 89 | 44 | 17 | 25 | 48 | 9 | 7 | 7 | 7 | **7** | 100% | 0% |
| 88 | 2 | 3 | 26 | 49 | 2 | 6 | 6 | 6 | **6** | 100% | 0% |
| 91 | 75 | 14 | 26 | 50 | 11 | 9 | 9 | 8 | **9** | 67% | 7% |
| 88 | 3 | 3 | 26 | 51 | 2 | 7 | 7 | 6 | **6** | 33% | 9% |
| 91 | 64 | 17 | 26 | 52 | 10 | 9 | 9 | 8 | **9** | 67% | 7% |
| 88 | 1 | 11 | 26 | 53 | 2 | 6 | 6 | 6 | **6** | 100% | 0% |
| 87 | 7 | 4 | 26 | 54 | 3 | 6 | 5 | 5 | **5** | 67% | 11% |
| 86 | 7 | 14 | 26 | 55 | 4 | 4 | - | 4 | **4** | 100% | 0% |
| 89 | 48 | 22 | 26 | 57 | 9 | 6 | 7 | 6 | **7** | 33% | 9% |
| 90 | 46 | 6 | 26 | 58 | 9 | 8 | 9 | 7 | **8** | 33% | 13% |
| 87 | 2 | 11 | 26 | 59 | 3 | 6 | 5 | 5 | **5** | 67% | 11% |
| 94 | 93 | 14 | 26 | 60 | 10 | 12 | 12 | 11 | **12** | 67% | 5% |
| 88 | 6 | 9 | 26 | 61 | 2 | 6 | 6 | 5 | **6** | 67% | 10% |
| 87 | 29 | 15 | 26 | 62 | 9 | 5 | 5 | 5 | **5** | 100% | 0% |
| 90 | 46 | 10 | 26 | 63 | 9 | 8 | 8 | 7 | **8** | 67% | 8% |
| 86 | 9 | 10 | 26 | 65 | 4 | 5 | 4 | 6 | **4** | 33% | 20% |
| 89 | 44 | 22 | 27 | 66 | 9 | 7 | 7 | 7 | **7** | 100% | 0% |
| 92 | 3 | 2 | 27 | 67 | 2 | 10 | 10 | 10 | **10** | 100% | 0% |
| 92 | 48 | 10 | 27 | 68 | 10 | 10 | 9 | 10 | **10** | 67% | 6% |
| 93 | 103 | 4 | 27 | 69 | 11 | 12 | 8 | 8 | **11** | 0% | 25% |
| 91 | 2 | 1 | 27 | 70 | 2 | 9 | 9 | 7 | **9** | 67% | 14% |
| 87 | 5 | 6 | 27 | 71 | 3 | 6 | 7 | 7 | **5** | 0% | 9% |
| 87 | 31 | 15 | 27 | 72 | 9 | 5 | 6 | 5 | **5** | 67% | 11% |
| 90 | 1 | 3 | 27 | 73 | 2 | 7 | 7 | 8 | **8** | 33% | 8% |
| 86 | 9 | 9 | 27 | 74 | 4 | 5 | 7 | 5 | **4** | 0% | 20% |
| 93 | 101 | 6 | 27 | 75 | 11 | 12 | 11 | 12 | **11** | 33% | 5% |
| 88 | 5 | 15 | 27 | 76 | 2 | 6 | 6 | 6 | **6** | 100% | 0% |
| 88 | 6 | 8 | 27 | 77 | 2 | 6 | 6 | 6 | **6** | 100% | 0% |
| 87 | 43 | 3 | 27 | 78 | 10 | 5 | 5 | 5 | **5** | 100% | 0% |
| 91 | 3 | 6 | 27 | 79 | 2 | 9 | 9 | 9 | **9** | 100% | 0% |
| 92 | 41 | 23 | 27 | 80 | 9 | 10 | 10 | 8 | **10** | 67% | 12% |
| 88 | 1 | 21 | 27 | 82 | 2 | 6 | 7 | 6 | **6** | 67% | 9% |
| 92 | 48 | 20 | 27 | 83 | 10 | 9 | 10 | 9 | **10** | 33% | 6% |
| 93 | 2 | 2 | 27 | 84 | 1 | 11 | 10 | 9 | **11** | 33% | 10% |
| 87 | 6 | 17 | 27 | 85 | 3 | 6 | 5 | 6 | **5** | 33% | 10% |
| 90 | 7 | 9 | 27 | 86 | 3 | 7 | 7 | 7 | **8** | 0% | 0% |
| 87 | 43 | 8 | 27 | 87 | 10 | 6 | 5 | 7 | **5** | 33% | 17% |
| 90 | 60 | 3 | 28 | 90 | 10 | 8 | 8 | 9 | **8** | 67% | 7% |
| 94 | 93 | 18 | 28 | 91 | 10 | 12 | 12 | 13 | **12** | 67% | 5% |
| 88 | 1 | 22 | 28 | 92 | 2 | 6 | 6 | 6 | **6** | 100% | 0% |
| 88 | 4 | 21 | 28 | 93 | 2 | 7 | 6 | 6 | **6** | 67% | 9% |
| 91 | 54 | 9 | 28 | 94 | 9 | 9 | 10 | 7 | **9** | 33% | 18% |
| 94 | 110 | 16 | 28 | 95 | 11 | 12 | 11 | 12 | **12** | 67% | 5% |
| 89 | 1 | 6 | 28 | 96 | 2 | 7 | 7 | 7 | **7** | 100% | 0% |
| 89 | 62 | 12 | 28 | 97 | 11 | 7 | 6 | 7 | **7** | 67% | 9% |
| 93 | 85 | 21 | 28 | 98 | 9 | 12 | 10 | 11 | **11** | 33% | 9% |

## Table 3 Continued

| Sample year | Sample no | Fish no | Fish length | Otol. no | Landing month | Broken/burnt otoliths<br>PA Spain Reader 2 | Unstained sliced section<br>PA Spain Reader 2 | Stained sliced sections<br>PA Spain Reader 2 | ACTUAL age | Percent agreement | Precision CV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 95 | 3 | 2 | 28 | 100 | 1 | 13 | 10 | 11 | **13** | 33% | 13% |
| 89 | 8 | 9 | 28 | 101 | 3 | 7 | 7 | 6 | **7** | 67% | 9% |
| 94 | 93 | 23 | 28 | 102 | 10 | 12 | 12 | 12 | **12** | 100% | 0% |
| 89 | 1 | 7 | 28 | 103 | 2 | 8 | 7 | 8 | **7** | 33% | 8% |
| 93 | 94 | 25 | 28 | 104 | 10 | 11 | 10 | 10 | **11** | 33% | 6% |
| 94 | 6 | 5 | 28 | 105 | 1 | 12 | 11 | 11 | **12** | 33% | 5% |
| 93 | 11 | 6 | 29 | 106 | 1 | 11 | 9 | 10 | **11** | 33% | 10% |
| 93 | 85 | 25 | 29 | 107 | 9 | 11 | 8 | 9 | **11** | 33% | 16% |
| 88 | 5 | 21 | 29 | 108 | 2 | 7 | 6 | 6 | **6** | 67% | 9% |
| 92 | 6 | 12 | 29 | 109 | 3 | 11 | - | 9 | **10** | 0% | 14% |
| 91 | 2 | 12 | 29 | 111 | 2 | 9 | 8 | 8 | **9** | 33% | 7% |
| 90 | 63 | 13 | 29 | 112 | 11 | - | 8 | 7 | **8** | 50% | 9% |
| 91 | 2 | 9 | 29 | 113 | 2 | 9 | 9 | 10 | **9** | 67% | 6% |
| 89 | 6 | 7 | 29 | 114 | 3 | 7 | 7 | 7 | **7** | 100% | 0% |
| 89 | 1 | 11 | 29 | 115 | 2 | 7 | 7 | 7 | **7** | 100% | 0% |
| 89 | 62 | 24 | 29 | 116 | 11 | 8 | 6 | 6 | **7** | 0% | 17% |
| 95 | 2 | 12 | 29 | 117 | 1 | 12 | 12 | 11 | **13** | 0% | 5% |
| 95 | 3 | 16 | 29 | 118 | 1 | 12 | 13 | 12 | **13** | 33% | 5% |
| 93 | 104 | 24 | 30 | 122 | 11 | 11 | 12 | 11 | **11** | 67% | 5% |
| 94 | 2 | 14 | 30 | 123 | 1 | 11 | 11 | 11 | **12** | 0% | 0% |
| 92 | 57 | 25 | 30 | 125 | 11 | 11 | 9 | 10 | **10** | 33% | 10% |
| 90 | 4 | 10 | 30 | 126 | 2 | 8 | 7 | 8 | **8** | 67% | 8% |
| 87 | 33 | 24 | 30 | 127 | 9 | 6 | 6 | 6 | **5** | 0% | 0% |
| 89 | 3 | 9 | 30 | 128 | 2 | 8 | 7 | 7 | **7** | 67% | 8% |
| 91 | 1 | 15 | 31 | 130 | 2 | 9 | 8 | 8 | **9** | 33% | 7% |
| 90 | 2 | 15 | 31 | 131 | 2 | 8 | 10 | 9 | **8** | 33% | 11% |
| 91 | 54 | 24 | 31 | 132 | 9 | 9 | 9 | 9 | **9** | 100% | 0% |
| 92 | 1 | 3 | 31 | 133 | 2 | 9 | 10 | 10 | **10** | 67% | 6% |
| 92 | 7 | 11 | 31 | 134 | 3 | 9 | 11 | 10 | **10** | 33% | 10% |
| 93 | 6 | 3 | 31 | 135 | 1 | 10 | 12 | 12 | **11** | 0% | 10% |
| 95 | 7 | 20 | 32 | 136 | 1 | 13 | 13 | 12 | **13** | 67% | 5% |
| 94 | 6 | 3 | 32 | 138 | 1 | 12 | 13 | 12 | **12** | 67% | 5% |
| 90 | 2 | 21 | 32 | 139 | 2 | 10 | 10 | 9 | **8** | 0% | 6% |
| 92 | 2 | 21 | 32 | 140 | 2 | 9 | 10 | 10 | **10** | 67% | 6% |
| 91 | 4 | 15 | 32 | 141 | 3 | 9 | 10 | 9 | **9** | 67% | 6% |
| 92 | 7 | 21 | 32 | 142 | 3 | 10 | 11 | 10 | **10** | 67% | 6% |
| 94 | 2 | 22 | 33 | 143 | 1 | 11 | 12 | 12 | **12** | 67% | 5% |
| 93 | 6 | 4 | 33 | 144 | 1 | 10 | 10 | 11 | **11** | 33% | 6% |
| 90 | 4 | 19 | 33 | 145 | 2 | 8 | 8 | 9 | **8** | 67% | 7% |
| 90 | 63 | 24 | 33 | 146 | 11 | 8 | 9 | 8 | **8** | 67% | 7% |
| 92 | 7 | 22 | 33 | 148 | 3 | 10 | 10 | 10 | **10** | 100% | 0% |
| 93 | 3 | 15 | 34 | 149 | 1 | 11 | 11 | 11 | **11** | 100% | 0% |
| 95 | 9 | 15 | 34 | 150 | 1 | 14 | 13 | 14 | **13** | 33% | 4% |
| 93 | 1 | 20 | 34 | 151 | 1 | 12 | 11 | 11 | **11** | 67% | 5% |
| 93 | 6 | 18 | 35 | 152 | 1 | 11 | 12 | 11 | **11** | 67% | 5% |
| 94 | 4 | 24 | 38 | 153 | 1 | 11 | 11 | 11 | **12** | 0% | 0% |
| | | | | | Total read | 131 | 132 | 134 | | **58.0%** | **9.3%** |
| | | | | | Total NOT read | 3 | 2 | 0 | | | |

**Table 4** By otolith-processing technique the number of age readings, the coefficient of variation (CV), the percent agreement and the absolute bias are presented by actual age for same age reader. By otolith-processing technique a weighted mean CV and a weighted mean percent agreement are given.

## SET L Comparison of otolith-processing techniques

| Nr AGE READINGS | | Broken/burnt otoliths | Unstained sliced sections | Stained sliced sections | |
|---|---|---|---|---|---|
| | ACTUAL age | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 | TOTAL |
| | 1 | 2 | 2 | 2 | 6 |
| | 2 | 5 | 5 | 5 | 15 |
| | 3 | 11 | 11 | 11 | 33 |
| | 4 | 15 | 16 | 17 | 48 |
| | 5 | 12 | 12 | 12 | 36 |
| | 6 | 12 | 12 | 12 | 36 |
| | 7 | 12 | 12 | 12 | 36 |
| | 8 | 12 | 13 | 13 | 38 |
| | 9 | 11 | 11 | 11 | 33 |
| | 10 | 12 | 11 | 12 | 35 |
| | 11 | 13 | 13 | 13 | 39 |
| | 12 | 9 | 9 | 9 | 27 |
| | 13 | 5 | 5 | 5 | 15 |
| Total | 1-13 | 131 | 132 | 134 | 397 |

| COEFF. of VARIATION | | Broken/burnt otoliths | Unstained sliced sections | Stained sliced sections | |
|---|---|---|---|---|---|
| | ACTUAL age | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 | ALL |
| | 1 | 47% | 47% | 0% | 17.3% |
| | 2 | 14% | 23% | 23% | 20.9% |
| | 3 | 20% | 10% | 10% | 9.6% |
| | 4 | 15% | 22% | 19% | 11.3% |
| | 5 | 9% | 20% | 16% | 10.6% |
| | 6 | 7% | 13% | 5% | 6.5% |
| | 7 | 8% | 6% | 9% | 5.7% |
| | 8 | 9% | 25% | 11% | 11.7% |
| | 9 | 3% | 23% | 11% | 10.6% |
| | 10 | 7% | 7% | 17% | 8.2% |
| | 11 | 6% | 13% | 11% | 8.6% |
| | 12 | 4% | 6% | 6% | 3.3% |
| | 13 | 7% | 11% | 10% | 6.4% |
| Weighted mean | 1-13 | 9.9% | 15.7% | 12.1% | 9.3% |
| | RANKING | 1 | 3 | 2 | |

| AGREEMENT % | | Broken/burnt otoliths | Unstained sliced sections | Stained sliced sections | |
|---|---|---|---|---|---|
| | ACTUAL age | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 | ALL |
| | 1 | 50% | 50% | 100% | 67% |
| | 2 | 0% | 60% | 60% | 40% |
| | 3 | 55% | 91% | 91% | 79% |
| | 4 | 60% | 69% | 59% | 63% |
| | 5 | 50% | 58% | 58% | 56% |
| | 6 | 75% | 67% | 92% | 78% |
| | 7 | 67% | 83% | 58% | 69% |
| | 8 | 75% | 31% | 31% | 45% |
| | 9 | 91% | 55% | 27% | 58% |
| | 10 | 50% | 55% | 67% | 57% |
| | 11 | 54% | 23% | 46% | 41% |
| | 12 | 67% | 44% | 44% | 52% |
| | 13 | 40% | 60% | 0% | 33% |
| Weighted mean | 1-13 | 60.3% | 57.6% | 56.0% | 57.9% |
| | RANKING | 1 | 2 | 3 | |

| ABSOLUTE BIAS | | Broken/burnt otoliths | Unstained sliced sections | Stained sliced sections | |
|---|---|---|---|---|---|
| | ACTUAL age | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 | ALL |
| | 1 | 0.50 | 0.50 | 0.00 | 0.33 |
| | 2 | 1.20 | 0.40 | 0.40 | 0.67 |
| | 3 | 0.27 | -0.09 | 0.09 | 0.09 |
| | 4 | 0.13 | 0.19 | 0.12 | 0.15 |
| | 5 | 0.50 | 0.08 | 0.67 | 0.42 |
| | 6 | 0.25 | -0.08 | -0.08 | 0.03 |
| | 7 | 0.17 | -0.17 | -0.25 | -0.08 |
| | 8 | 0.00 | -0.38 | -0.08 | -0.16 |
| | 9 | -0.09 | -0.55 | -0.73 | -0.45 |
| | 10 | -0.17 | 0.09 | 0.08 | 0.00 |
| | 11 | 0.15 | -0.69 | -0.54 | -0.36 |
| | 12 | -0.33 | -0.33 | -0.33 | -0.33 |
| | 13 | -0.20 | -0.80 | -1.00 | -0.67 |
| Weighted mean | 1-13 | 0.14 | -0.17 | -0.10 | -0.05 |
| | RANKING | 2 | 3 | 1 | |

| Overall ranking | Broken/burnt otoliths | Unstained sliced sections | Stained sliced sections |
|---|---|---|---|
| | PA Spain Reader 2 | PA Spain Reader 2 | PA Spain Reader 2 |
| Ranking Coefficient of Variation | 1 | 3 | 2 |
| Ranking Percentage Agreement | 1 | 2 | 3 |
| Ranking Absolute bias | 2 | 3 | 1 |
| OVERALL RANKING | 1 | 3 | 2 |

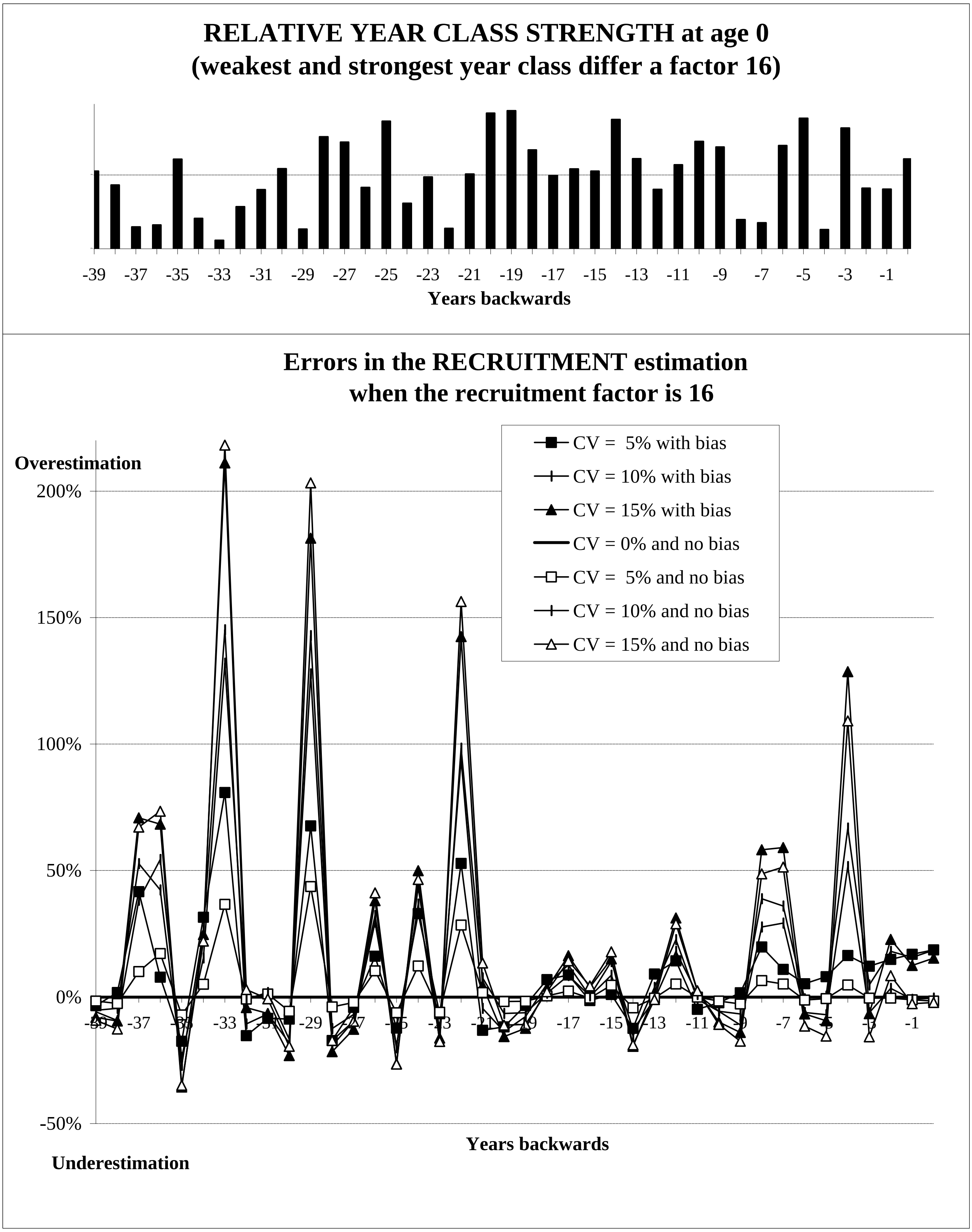


**Figure 1** Effect of age reading errors on the assessement of horse mackerel when the recruitment factor is 16.
<u>Upper panel</u>: Input of recruitment for the calculation of the true population and the catch at age.
<u>Lower panel</u>: Errors in recruitment estimation, when age reading errors occur in the catch in number data.
(from the addendum of ICES, 1999)

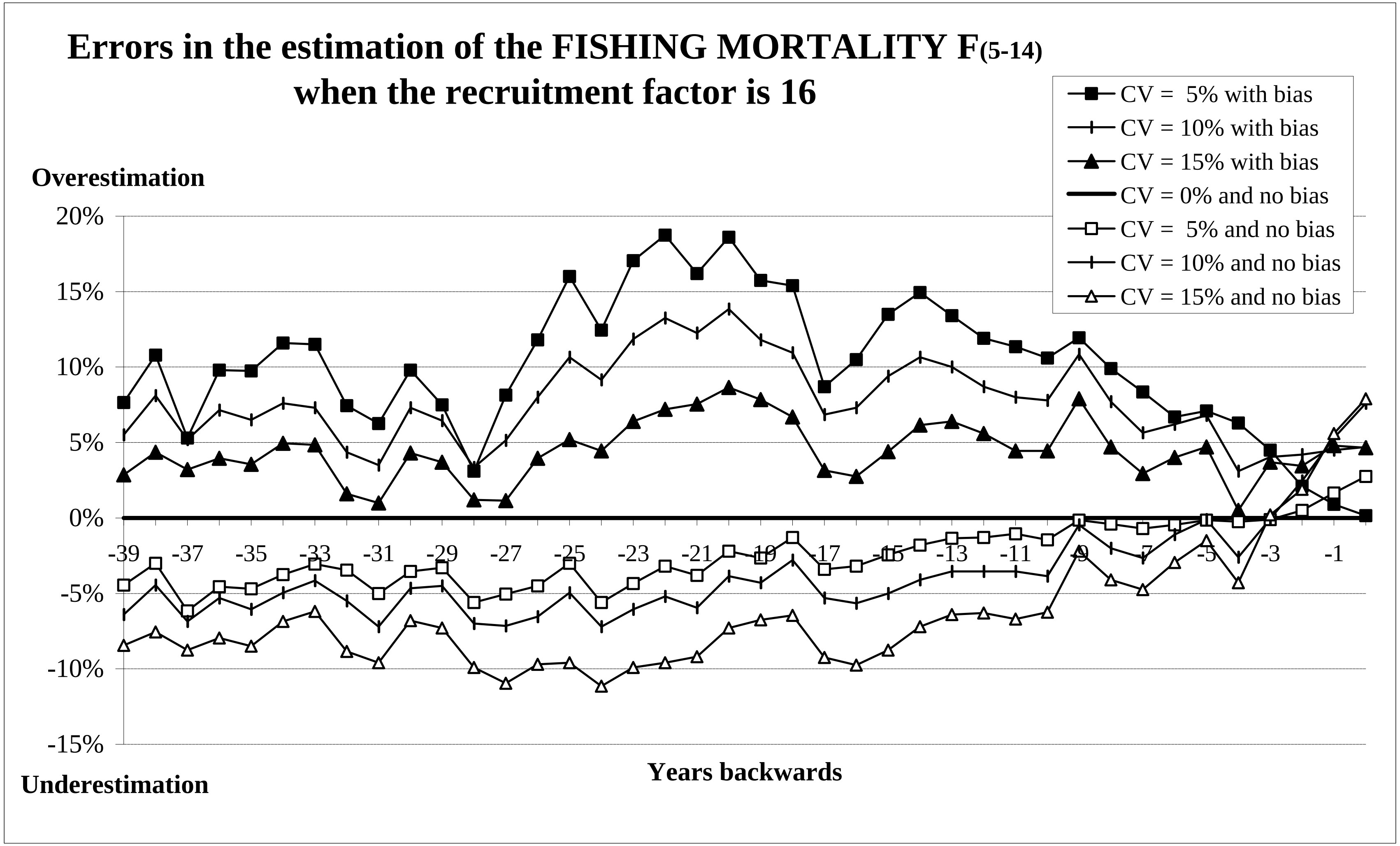


**Figure 2** Errors in the estimation of fishing mortality, when age reading errors occur in the catch in number data (from addendum of ICES, 1999).

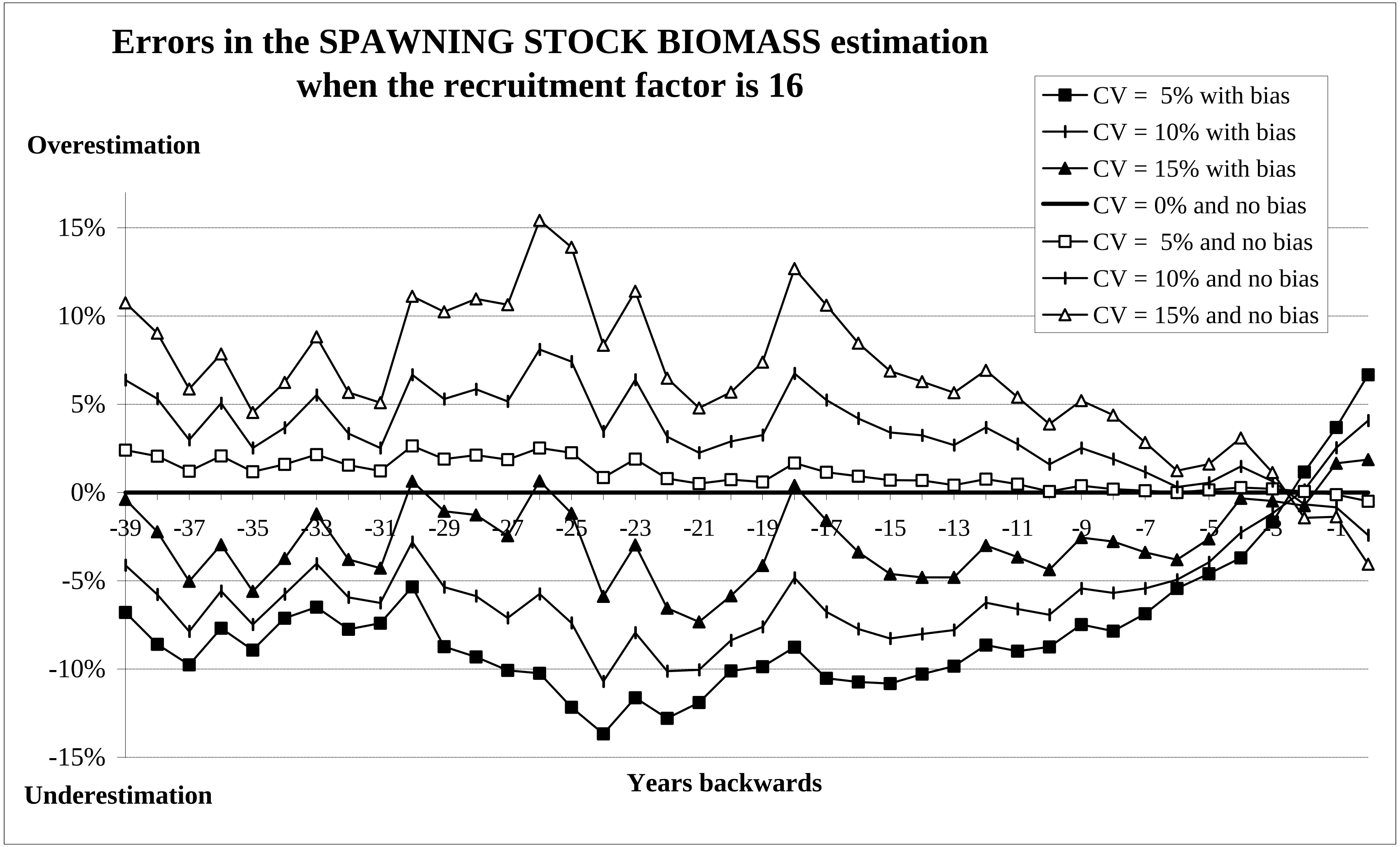


**Figure 3** Errors in the estimation of spawning stock biomass, when age reading errors occur in the catch in number data (from addendum of ICES, 1999).

# Otolith SET K Comparison of otolith-processing techniques

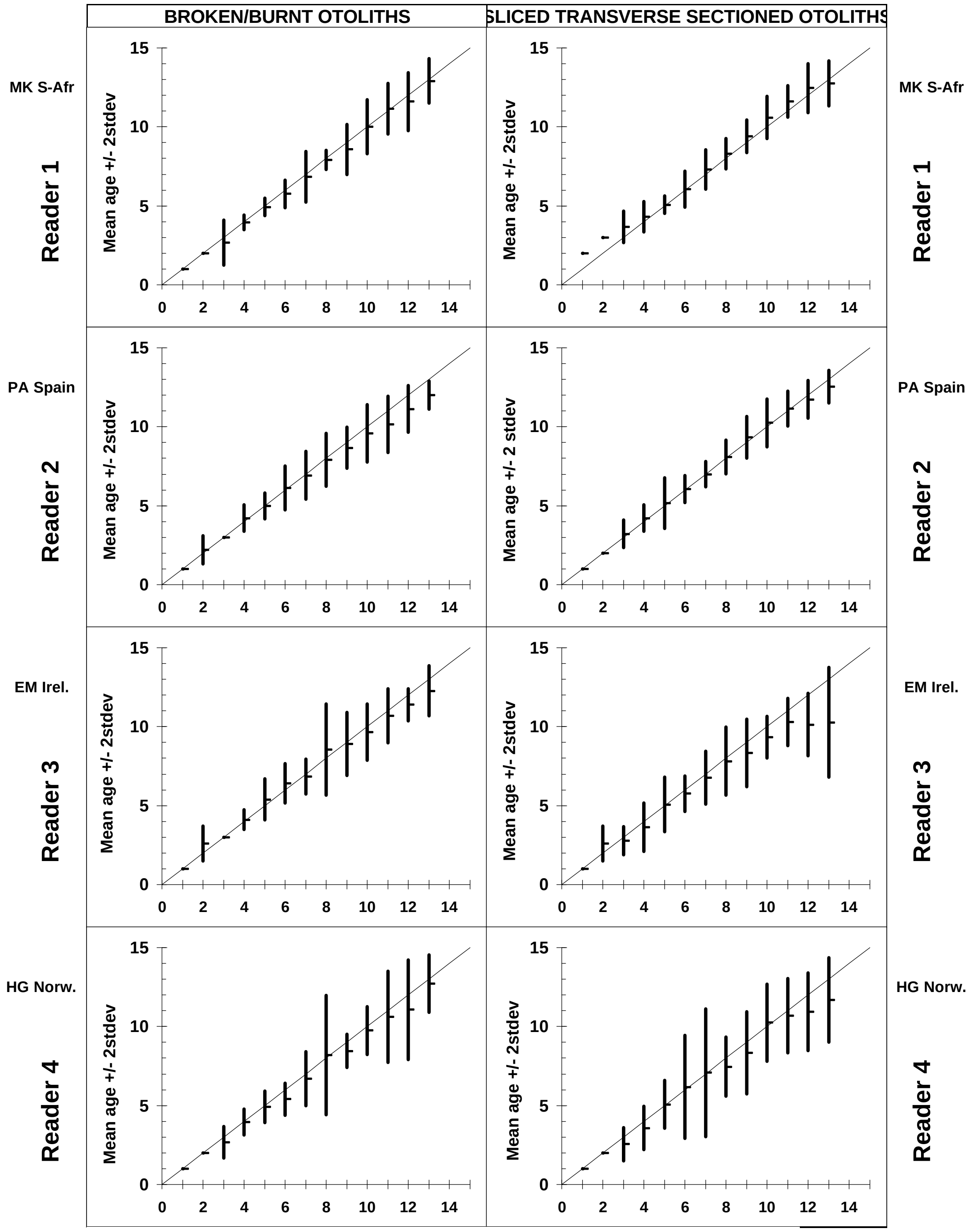


**Figure 4** In the age bias plots above the mean age recorded +/- 2stdev of each age reader is plotted against the actual age. The estimated mean age corresponds to actual age, if the estimated mean age is on the 1:1 equilibrium line (solid line). Of each fish one otolith is prepared according the broken/burnt otolith processing method (figures left column) and the other otolith is prepared according the sliced transverse section method (figures right column).

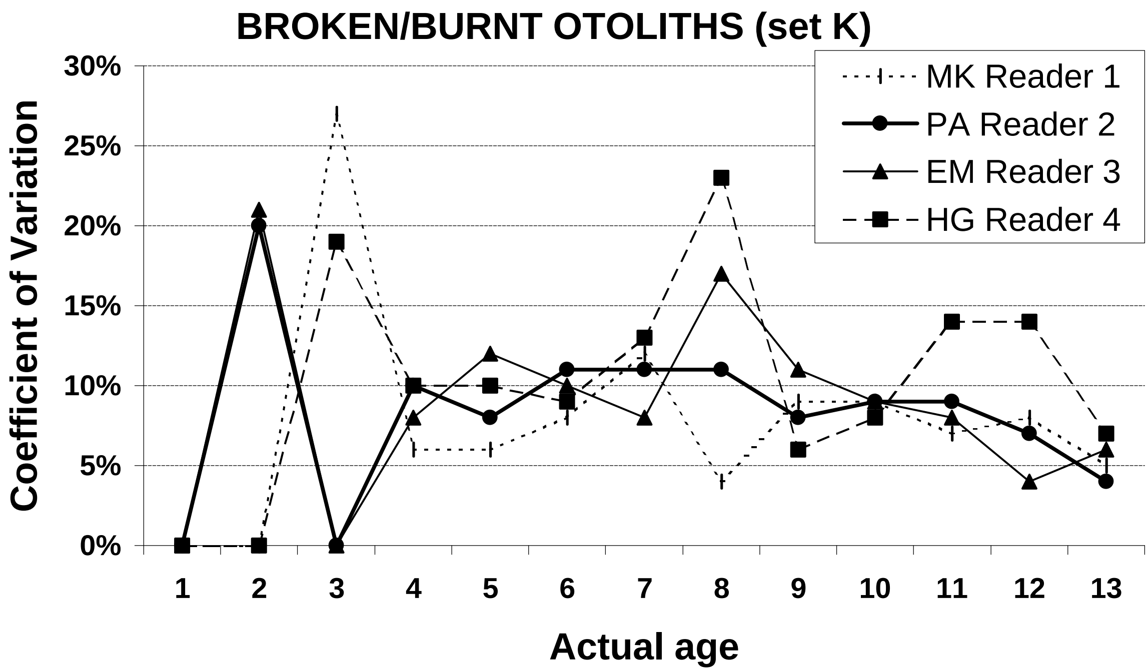


**Figure 5A** The precision in age reading (coefficient of variation) by actual age by age reader from the broken/burnt otoliths of set K.

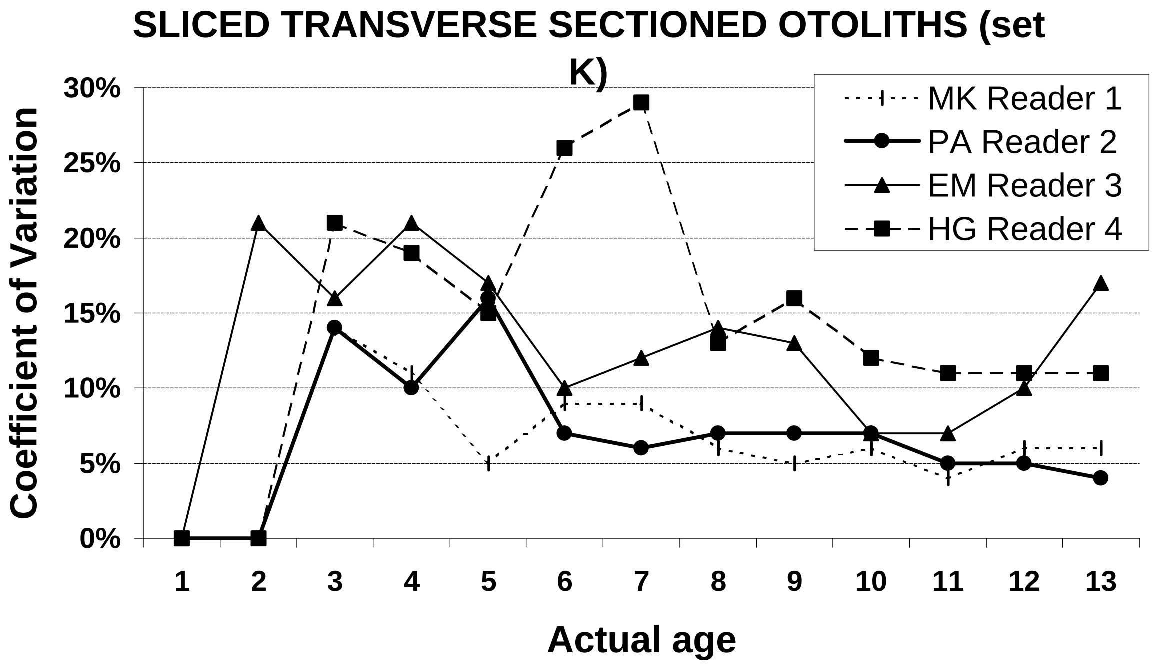


**Figure 5B** The precision in age reading (coefficient of variation) by actual age by age reader from the sliced transverse sectioned otoliths of set K.

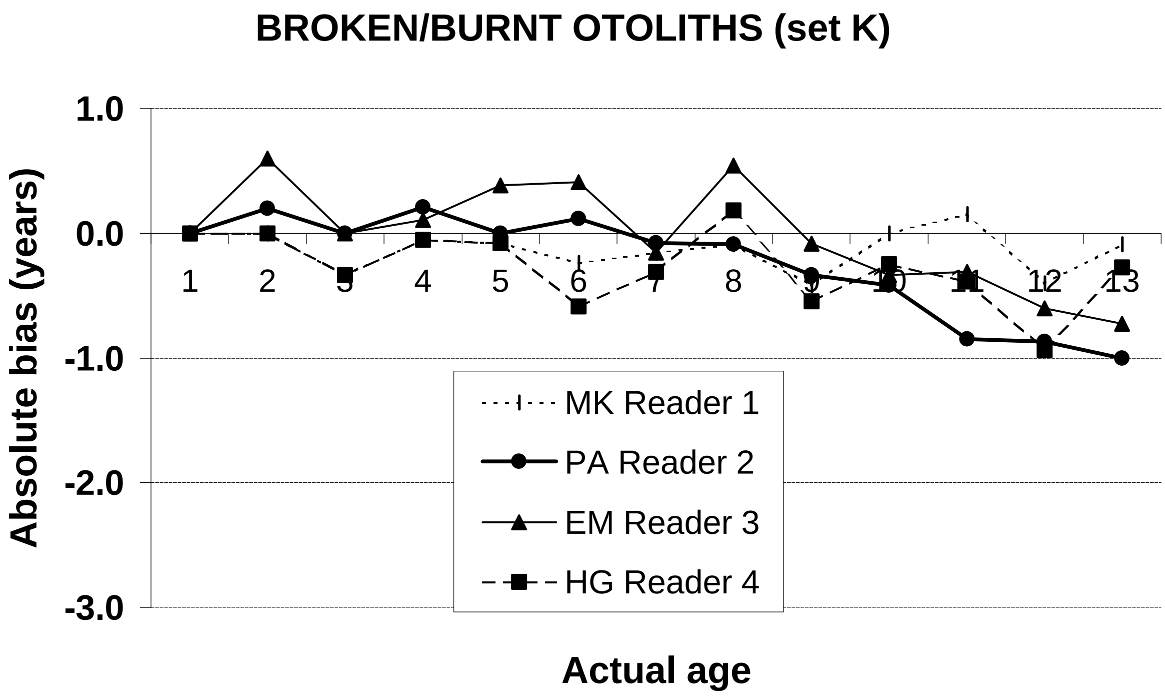


**Figure 6A** The absolute bias (years) by actual age by age reader from the broken/burnt otoliths of set K.

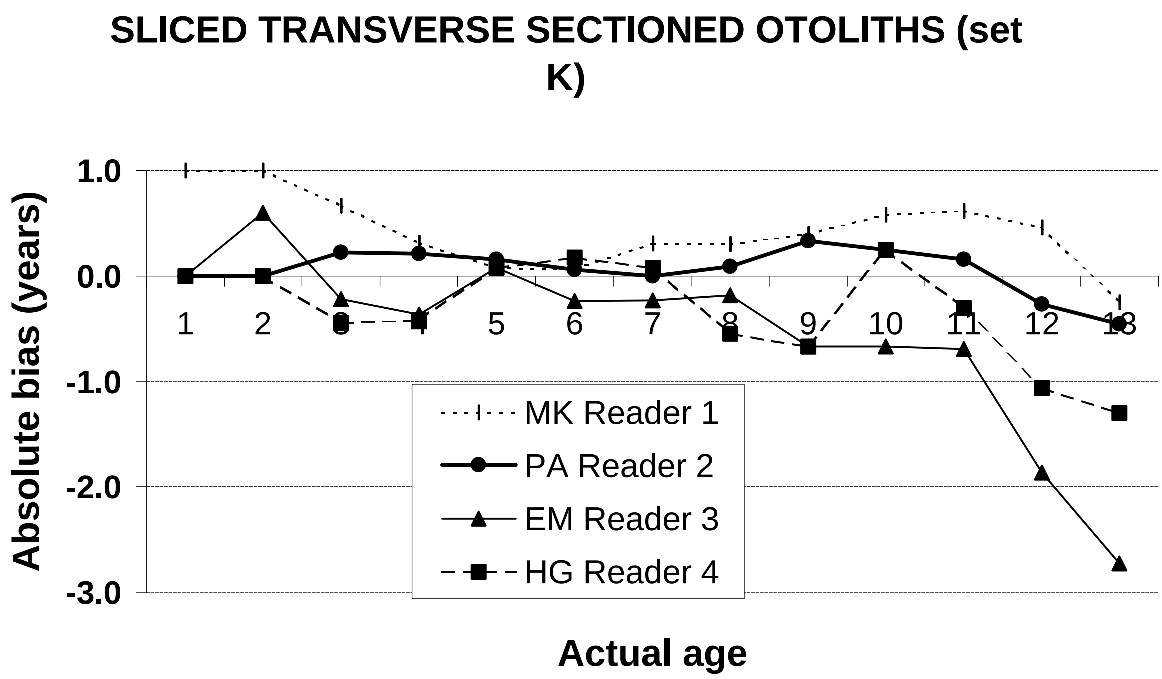


**Figure 6B** The absolute bias (years) by actual age by age reader from the stained sliced transverse sectioned otoliths of set K.

**Otolith SET L    Comparison of otolith-processing techniques**

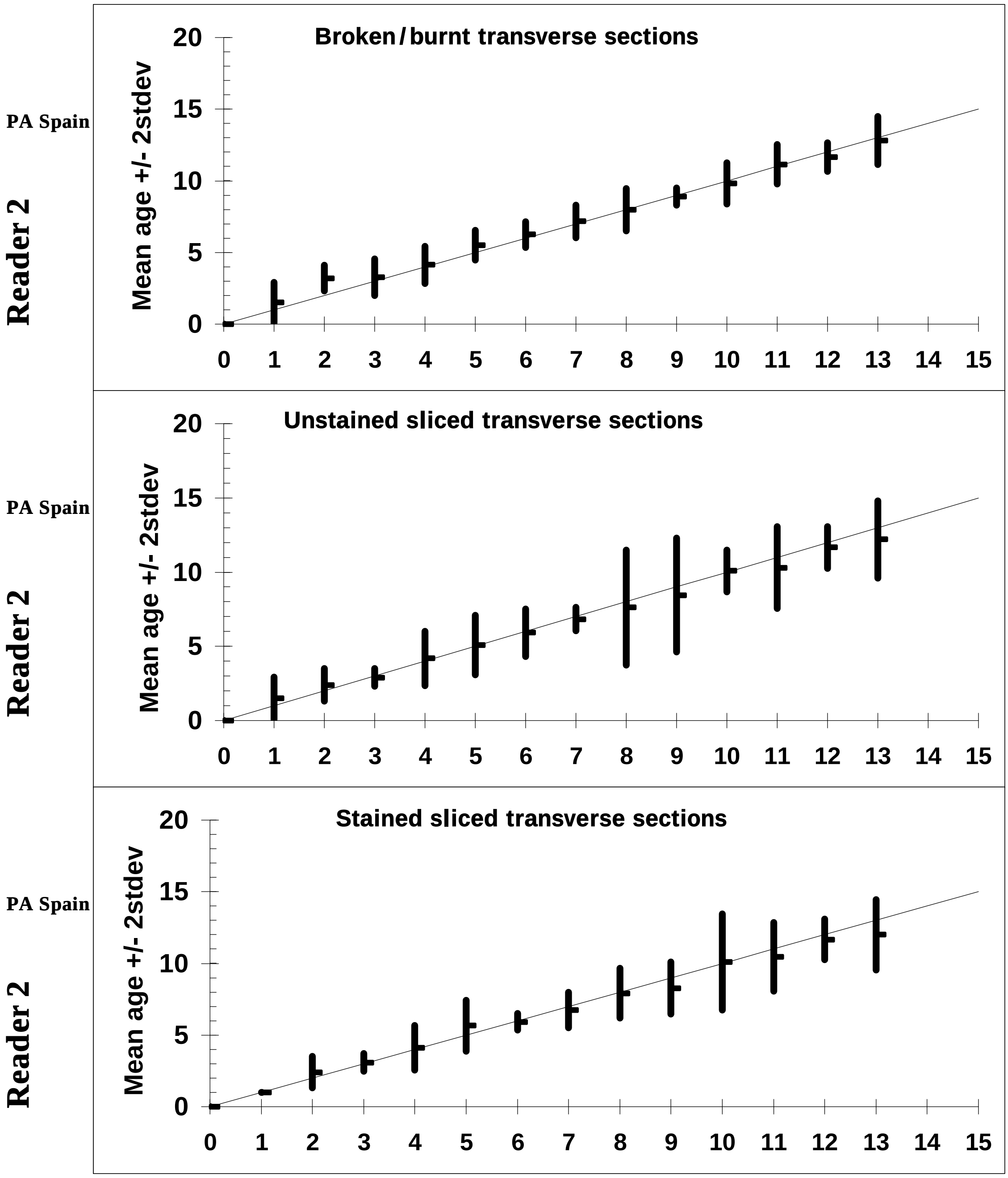


**Figure 7** Of each fish one otolith is prepared according the broken/burnt processing method and the other otolith according the sliced transverse section method (reading both before and after staining with Neutral Red). In the age bias plots above the mean age recorded +/- 2stdev of the same age reader are plotted against the actual age. The estimated mean age corresponds to actual age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

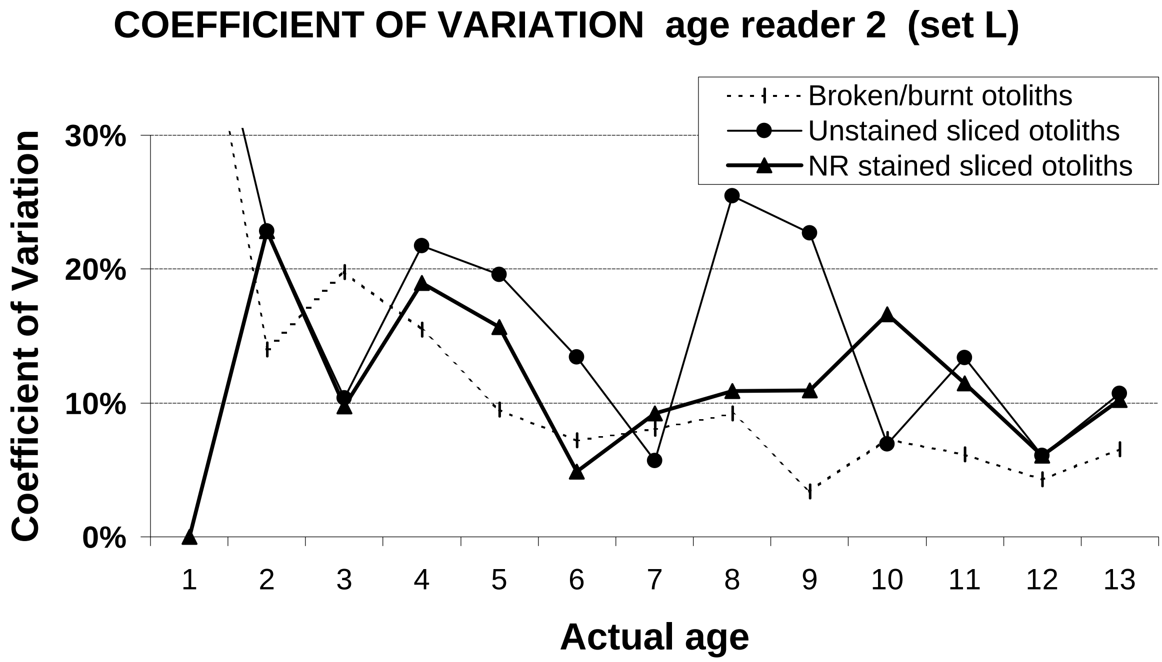


**Figure 8** The coefficient of variation by actual age for age reader 2 by otolith processing technique for otolith set L.

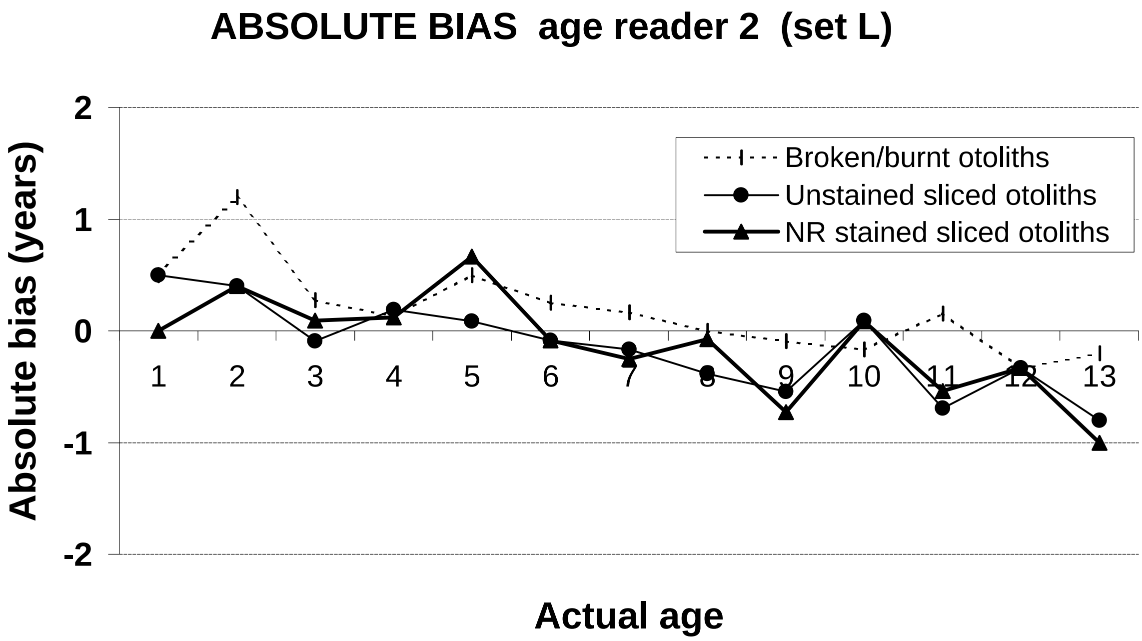


**Figure 9** The absolute bias (years) by actual age for age reader 2 by otolith processing method for otolith set L.