BENEFIT

BENGUELA ENVIRONMENT FISHERIES INTERACTION & TRAINING PROGRAMME

Report of the second horse mackerel otolith reading workshop

12 – 28 November, 2001

NatMIRC, Swakopmund, Namibia

BENEFIT Secretariat

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SUMMARY

The Second BENEFIT Horse Mackerel Otolith Reading Workshop was convened at NatMIRC in Swakopmund, Namibia from 12 to 28 November 2001. The workshop was attended by participants from all three countries in the Benguela Region (Angola, Namibia and South Africa) as well as an expert in the field, Dr. Michael Kerstan. The workshop was convened with the intention of:

- Assessing the suitability of a new method of otolith preparation in terms of the readability of the resulting specimens for age estimation purposes.
- Providing further training in the development of stable otolith interpretation criteria.
- Assessing the age estimation performance of the researchers in terms of the precision and stability with which they interpreted the specimens.
- Providing management with preliminary age length keys if the results of the analyses indicate satisfactory levels of precision and stability.

In an effort to extend the workshop to represent the entire Benguela region, otoliths of Cape horse mackerel from South African and Namibian waters, as well as Cunene horse mackerel from Angolan waters were analyzed during the workshop. In accordance with the recommendation of the first BENEFIT horse mackerel otolith reading workshop (June 2000), a new, unified method of preparing otoliths was implemented during this 2001 workshop, with all otoliths being prepared for examination by burning and slicing. Disregarding various technical problems, the method was found to produce specimens of generally satisfactory readability in all three groups of otoliths. The relatively poor readability apparent in the South African specimens was attributed largely to the variable environmental conditions experienced by these fish, rather than any technical inadequacies of the method.

Training in otolith reading, with the intention of stabilizing the otolith interpretation criteria employed by the participants, was provided by Dr. Kerstan during group discussions using an image analysis system. The performance of the participants, in terms of the consistency with which they interpreted the otoliths, was assessed using multiple readings of the same otolith sets. With the exception of the South African otoliths, the participants generally displayed satisfactory levels of precision as individuals, indicating that their interpretation criteria were suitably stable. As a group, however, the participants were too imprecise to permit the generation of consistent, comparable age length keys for any of the otolith sets. This result indicates that the interpretation criteria employed by each participant generally differed substantially from those employed by the other participants. This has profound implications for the comparability of age estimates, and hence the management of shared stocks. This also indicates that when more than one of the participants are employed in the same institution (e.g. Namibia), the readers would generate different age length keys from the same otolith samples. The use of the age estimates obtained during this workshop for age length keys is consequently not recommended. Other recommendations arising from this workshop:

- The participants require further training aimed at stabilizing and standardizing their interpretation criteria.
- Technical aspects of otolith preparation were not satisfactory, primarily because different technicians were involved in preparing the otoliths. Equipment and training inadequacies also contributed to this shortcoming. Improvements aimed at enhancing the efficiency and success rate of the method are provided.
- The issue of personnel fluctuations (otolith readers and technical staff) has to be addressed. Both sectors are extremely valuable personnel, and should be retained within the age estimation program at all costs to ensure continuity in the results.
- A follow-up workshop aimed at generating a reference otolith collection should be convened. This material would be an invaluable asset for the region, as researchers could use these otoliths to calibrate and stabilize their interpretation criteria.

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2. INTRODUCTION

Stocks of the two species of horse mackerel that occur in the Benguela region, namely Cape (*Trachurus trachurus capensis*) and Cunene horse mackerel (*Trachurus trecae*) are being exposed to increasing levels of commercial exploitation, highlighting the requirement for effective management measures to ensure their sustainable utilization. Such measures can only be implemented with confidence if they are based on reliable stock assessment procedures. Size-structured stock assessment models have been found to be unsuitable for Southern African horse mackerel stocks, and the decision has recently been made to move to an age-structured VPA approach. A fundamental requirement for this approach to stock assessment is the availability of robust information concerning the age structure of the population(s). This requirement has been highlighted in the BENEFIT Science Plan, and more recently during the International Workshop on the Research and Management of Horse Mackerel in Namibian Waters convened in Swakopmund, Namibia (26 – 30 March, 2001). The lack of representative age length keys for horse mackerel in the Benguela has been identified as a major shortcoming of current stock assessment models employed in the region (the age length key currently used for Namibian horse mackerel dates back to 1987).

To overcome this obstacle, effort has been directed towards developing an age estimation program for horse mackerel using periodic features in sagittal otoliths. The first step in implementing such a program is to develop the best method of preparing, examining and analyzing otoliths. Once the method been developed, it has to be validated. The validation process is comprised of two phases, the first testing the accuracy of the age estimates obtained using the method, and the second testing the precision and consistency with which researchers obtain the age estimates. The first phase of validating age estimation from horse mackerel otoliths is the subject of an otolith-marking project currently being conducted at the Marine and Coastal Management Research Aquarium in Cape Town, South Africa, under the auspices of BENEFIT.

The development of a suitable method for horse mackerel age estimation, and the subsequent assessment of the precision with which the age estimates are obtained was the subject of the first BENEFIT Horse Mackerel Age Determination Workshop convened in Swakopmund in June 2000. The objective of that workshop was to facilitate the development and implementation of a reliable horse mackerel age estimation program in the Benguela region, and build capacity for this purpose by:

- Gaining knowledge and experience of methods used for preparing otoliths for age estimation, with the intention of developing an effective method of preparing otoliths of Cape horse mackerel for this purpose
- Assisting researchers in developing a stable set of criteria for interpreting otoliths, thereby ensuring consistent and precise age estimation by each researcher
- Promoting agreement in otolith interpretation between readers and institutions

Otoliths of Cape horse mackerel from Namibian waters were examined by five different readers, and the results assessed. The major conclusions and recommendations arising from June 2000 workshop were:

- Further training directed at developing stable otolith interpretation criteria is critical.
- An otolith exchange program should be implemented to facilitate this process.
- The use of untreated, as well as burnt and broken otoliths for age estimation is not satisfactory, and a new method should be developed.
- A method involving burning and subsequent slicing of otoliths should be implemented and assessed during a follow-up workshop.
- Efforts should be made to ensure that personnel trained to read otoliths are retained in the program.
- Otoliths of Cunene horse mackerel appear to be unsuitable for age estimation purposes, and methods employing other structures should be explored.

In accordance with these recommendations, and considering the urgent requirement of management for horse mackerel age length keys, the workshop described in this report was convened in Swakopmund in November 2001, with the specific objectives of:

1. Using a new method to prepare otoliths of horse mackerel for age estimation purposes, specifically to burn and slice otoliths.
2. Assess the suitability of the new method in terms of the readability of the resulting specimens.
3. Provide further training in the development of stable otolith interpretation criteria to researchers involved with horse mackerel age estimation
4. Assess the age estimation performance of the researchers in terms of the precision and stability with which they interpreted the specimens.
5. Provide management with preliminary age length keys if the results of the analyses indicate satisfactory levels of precision and stability.

An initial objective of the workshop was also to conduct a preliminary indirect age validation analysis using the results of the age estimation exercise. Unfortunately, time constraints precluded the participants from reading sufficient numbers of otoliths within each age group to ensure a statistically valid analysis. The validation objective was consequently excluded from the activities of the participants.

An expert in the field, Dr. Michael Kerstan was invited to participate in this workshop. Dr. Kerstan's contribution to the workshop was as follows:

- To provide training in the reading of otoliths of horse mackerel from Namibian and Angolan waters, and specifically to assist the participants in developing stable criteria for the recognition/identification of the structural features in the otoliths of horse mackerel used to estimate the age of individuals.
- To assist in the modification and/or standardization of existing otolith preparation methods where required
- To assist with analyses of the precision and bias of the age estimates obtained by the participants from 3 replicate readings of approximately 300 otoliths
- Assist with the final evaluation of all components
- Provide recommendations

In an effort to extend the workshop to represent the entire Benguela region, otoliths of Cape horse mackerel from South African and Namibian waters as well as Cunene horse mackerel from Angolan waters were included in the age reading exercise. In accordance with the recommendation of the 2000 workshop, a unified method of preparing otoliths was implemented during this 2001 workshop. All otoliths from Namibia, South Africa and Angola were prepared for examination by burning and slicing.

It should be emphasized that this workshop did not attempt to assess the issue of accuracy of the age estimates. In the absence of otoliths from fish of known age, the accuracy (or conversely, error) of age readings cannot be estimated. There are no otoliths from Benguela horse mackerel of known-age currently available to use for this purpose. The aim of the workshop was rather to assess the stability of otolith interpretation criteria employed by the participants, with the ultimate objective of standardizing horse mackerel age estimation procedures in the region. When conducting a large-scale age estimation program that will continue over several years (such as is the case when generating age length keys for stock assessment purposes), it is imperative that the reader(s) display satisfactory levels of precision and stability and minimal bias in their interpretation of otolith structure over time. In other words, the age assigned to an individual fish by a reader will be the same (or similar, within specified limits) to that assigned by the same reader to the same individual during later readings. Bias measures consistent trends to over- or underestimate the age of the fish, either relative to the true age (absolute bias, Eltink et al., 2000), or relative to sequential age estimates obtained by a single reader (within-reader relative bias), or relative to another reader(s) (among-reader relative bias).

3. WORKSHOP SCHEDULE

Day 1 (Monday 12 November 2001)

Welcome by the chairperson (Frances Dealie) and the opening discussion.

Present: Frances Dealie (MFMR, Namibia)
Michael Kerstan (Independent consultant)
Henriette Lutuba (IIP, Angola)
Vicky Herbert (MFMR, Namibia)
Deon Durholtz (BENEFIT)
Angie Kanandjembo (MFMR, Namibia)
Dave Boyer (MFMR, Namibia)
Jens Otto Krakstad (MFMR, Namibia).

The discussion was opened by Dr. Michael Kerstan, who stressed that the primary objective of the workshop was to stabilize the criteria employed by researchers to interpret otolith structure and estimate the age of individuals. This would be assessed by checking for 'drift' in the age estimates produced by the participants.

Dave Boyer questioned whether the otolith slicing procedure would have to remain in place, considering that it is time consuming and labour intensive. He further questioned whether it would be possible to use a sub-set of sliced otoliths to 'back-validate' to whole otoliths. Michael Kerstan stated that the slicing procedure had to remain in place to avoid problems with reader interpretation and 'drift'. Whole otoliths should be available (particularly in younger fish) to assist with the distinction between false rings and annuli.

Dave Boyer then raised the question of whether any validation would be attempted during the workshop. Michael Kerstan felt that it might be possible to use MULTIFAN to indirectly validate the age estimates by comparing age length keys with size-frequency distributions. This would, however, have to be considered only as preliminary validation, and it would be unlikely that sufficient numbers of specimens could be analyzed within the time available for the workshop. He further stressed the importance of consistent age estimation throughout the Benguela region, stating that validation was currently of secondary importance.

Angie Kanandjembo asked when Frances Dealie would be able to begin age estimation of Namibian horse mackerel. Michael Kerstan replied that the results of this workshop would have to be assessed before that decision could be made. Meaningful age estimates could only be produced if the reader displayed acceptable levels of consistency in their interpretation of otoliths.

Dave Boyer asked whether or not Frances needed help with ageing. Michael responded that a team of researchers actively involved in age estimation was a valuable asset, in that knowledge and experience are retained within the institution. He provided an example of a laboratory in Nanaimo, Canada where 6 technicians were involved. He further stated that within such a team, it is preferable to have all members equally trained and experienced, because then personalities play a role in influencing the process. It would be best to have one very well trained and experienced person who then trains the others. Dave mentioned the difficulties associated with building a team of this nature, and stated that all personnel involved in age estimation should have been invited to the workshop. The problem of staff fluctuations was raised by Michael in answer to this.

Jens Otto Krakstad questioned the approach of using otolith weight to estimate age. Michael responded by saying that this was a possibility for age groups of up to 6 or 7 years, but not in older age groups (which are the critical ones). He further stated that if this approach is adopted, all otoliths have to be sampled at the same time of year.

Dave Boyer stressed the importance of this workshop by putting it in perspective with regard to the management of Namibian horse mackerel. Length-based VPA techniques are in the process of being discarded as unsuitable for horse mackerel stock assessment, and age-based methods are currently being implemented. Age length keys are critical for this purpose.

Day 2 (Tuesday 13 November 2001)

Group discussion using the image analysis system (IAS) and preparations of Namibian horse mackerel otoliths (burnt, embedded and sliced). Discussion was directed at the clarification of criteria regulating the identification of annuli and annual growth zones (AGZ's). This was crucial to any further age estimation activities because a new method of otolith preparation was being used for the workshop, namely otolith slices (see section 4). The previous workshop had used a number of different techniques such as untreated otoliths, burnt otoliths, or burnt and broken otoliths. Since the criteria for annulus recognition vary depending on the preparation technique, the participants had to clarify the criteria applicable to otolith slices. During this session, it was observed that many of the Namibian specimens had been insufficiently burnt during preparation, resulting in poor contrast between the opaque and hyaline zones. The available specimens were subsequently assessed, and those suitable for accurate age estimation purposes selected for the workshop. Only preparations from fish larger than 30 cm total length were selected for this purpose. During this process, technical assistants continued preparing further Namibian horse mackerel otoliths.

Day 3 (Wednesday 14 November 2001)

Continued the selection process on specimens from Namibia that had been prepared the previous day. Once this was complete, the selected otoliths were used for further group discussions concerning annulus identification criteria. The technical assistants began preparing otoliths of South African *T. capensis* and Angolan *T. trecae* that had been made available by M&CM (South Africa) and IIP (Angola).

Day 4 (Thursday 15 November 2001)

Prepared images and printouts of selected otoliths that were considered to be suitable as demonstration specimens for the various annulus identification criteria established by the participants. These were used for further group discussions to clarify identification criteria.

The participants began the age estimation process. All participants completed the first reading of the set of Namibian *T. capensis* otoliths.

Day 5 (Friday 16 November 2001)

Michael Kerstan had conducted a preliminary assessment of the results of the previous day's otolith reading, and had detected several problems. The distinction between "true" annuli and false rings was still presenting problems for several of the participants. Group discussions using the IAS attempted to resolve these interpretation problems. An additional problem evident in the data was that there was some confusion among the participants as to whether the estimates should reflect simple counts of the number of annuli, or represent year class allocations. Considering that the primary objective of horse mackerel age estimation in the Benguela region is to provide age length keys for use in stock assessment models, the participants agreed that the data should allow year class allocations for each individual, and that the "winter to winter" year class convention should be adopted for this purpose. Once these concepts had been clarified (see section 4.4. for a detailed discussion), the results of the first otolith reading were discarded and the participants conducted another reading (considered to be the first reading) of the Namibian specimens.

Day 6 (Saturday 17 November 2001)

The participants completed a second reading of the Namibian otolith set.

Day 7 (Sunday 18 November 2001)

The third reading of the Namibian otolith set was completed.

Day 8 (Monday 19 November 2001)

MK had established that inconsistencies in the results of the three readings indicated that some confusion concerning the year class allocation process was still apparent. The procedure was again discussed in order to resolve the problems. Inconsistencies in the results were subsequently corrected. MK conducted several more readings on selected Namibian otolith specimens in an attempt to improve his percent agreement level. Since the technical assistants were no longer available, the other participants continued preparing the South African *capensis* and Angolan *trecae* otoliths.

Day 9 (Tuesday 20 November 2001)

The participants continued preparing the South African and Angolan otolith specimens. MK began preliminary data analysis.

Day 10 (Wednesday 21 November 2001)

Results of the preliminary data analysis were presented by MK and discussed by the group. The participants conducted the first reading of the South African otolith set.

Day 11 (Thursday 22 November 2001)

Results obtained during the first reading of the South African otoliths indicated that several of the participants were still having difficulties with the concept of year class allocation, specifically with respect to the assessment of the width of the marginal increment and the implication of the catch date. Further discussions were required to resolve the issue, after which the data were corrected where necessary. The participants conducted a second reading of the South African otolith set.

Day 12 (Friday 23 November 2001)

The participants completed a third reading of the South African otolith set, and subsequently completed the preparation of the Angolan otolith set.

Day 13 (Saturday 24 November 2001)

The Angolan otoliths were assessed, and satisfactory specimens selected for age reading. All participants subsequently conducted a first reading of these otoliths.

Day 14 (Sunday 25 November 2001)

The participants completed second and third readings of the Angolan otolith set.

Day 15 (Monday 26 November 2001)

MK and DD entered the data into a spreadsheet, and established that several specimens had to be re-read by the participants (those specimens that were considered unreadable during one of the reading sessions, but then considered readable in subsequent sessions). These specimens were re-read where necessary. MK and DD continued preliminary evaluations of the results, discussed these with the other participants, and proceeded with producing hard copies for this report.

Day 16 (Tuesday 27 November 2001)

Continued the data analyses and printing of figures and tables for the report. All participants then discussed the results, and began compiling the report, concentrating on recommendations and the direction of future research.

Day 17 (Wednesday 28 November 2001)

Final discussion of the results of the workshop and recommendations to be included in the report. The chairperson closed the workshop, and the visiting participants left Namibia.

4. MATERIALS AND METHODS

4.1. Terminology (definitions employed during this workshop):

Otolith: Sagittal otolith of a fish

Opaque (zone): Zone of enhanced calcification. Appears as white in colour when the otolith is viewed with reflected light against a dark background, or as a pale yellow or brown colour when the otolith has been burnt. Appears as a broad, dense (opaque) zone when an untreated otolith is viewed with transmitted light. Visible as a relatively light, transparent zone in a burnt otolith viewed with transmitted light.

Hyaline (zone): Zone of reduced calcification. Appears as dark gray when the otolith is viewed with reflected light against a dark background, or as a dark brown colour when the otolith has been burnt. Appears as a light, translucent zone when an untreated otolith is viewed with transmitted light. Visible as a dark, opaque zone when a burnt otolith is viewed with transmitted light.

Annual growth zone (AGZ): Zone presumed to have been deposited over the course of one calendar year. Consists of a relatively broad, opaque zone with (possibly) multiple hyaline zones, but with one, clearly defined annulus (hyaline zone) at it's margin.

Annulus: A clearly defined hyaline zone demarcating the end of an annual growth zone.

False ring: A hyaline zone/ring that resembles an annulus, but is generally narrower, and not continuous around the otolith.

Margin: The outer perimeter of the otolith, or the outer edge of an annual growth zone.

Marginal increment: The part of the otolith between the last, complete annual growth zone and the perimeter of the otolith (i.e. the region outside the last annulus deposited on the otolith).

Age (true age): The number of years (expressed as a whole number) lived by a fish before capture.

Precise age: The number of years lived by a fish before capture, expressed with a resolution of 0.1 years. Calculated by comparing the width of the marginal increment with the expected width of the corresponding annual growth zone.

Modal age: Represents the most frequently occurring age estimate in a series of age estimates for a given otolith, obtained either from multiple readings by given reader, or from multiple readings by a number of different readers. The "most often agreed upon age".

Mean age: The average of a series of age estimates for a given otolith obtained either from multiple readings by given reader, or from multiple readings by a number of different readers.

Age group: Refers to a group of fish that are all of the same age (expressed in years) regardless of when they were hatched.

Year class: Refers to all fish that were hatched in a particular year, and specifically all fish that hatched between 1 July of a given year and 30 June of the following year (assuming the "winter – to winter" year class convention generally adopted for the southern hemisphere). For example, the 1995 / 1996 year class comprises all fish that hatched between 1 July 1995 and 30 June 1996. With the passage of time, fish from this year class will move into progressive age groups as they grow older, but will always remain in the same year class.

Accuracy: The degree to which an age estimate conforms to the true age of the fish.

Precision: A measure of the degree with which a series of age estimates obtained from a given otolith conform to each other. Has nothing to do with accuracy. A series of age estimates could be very precise (i.e. very similar to each other), but greatly inaccurate (i.e. very different to the true age of the fish).

Bias: A directional trend in the error of a series of age estimates.

Absolute bias: Measures the tendency for a series of age estimates to deviate from the true age of a fish in a given direction and by a specified amount.

Relative bias: Measures the tendency of a series of age estimates to deviate from some reference age such as modal or mean age. The latter is usually employed when otoliths from fish of known age are not available (i.e. no data for true age), and a reference age computed from multiple estimates by one or more readers is used instead.

4.2. Otolith samples:

Otoliths from Cape horse mackerel (*Trachurus trachurus capensis*) collected in both Namibian and South African waters, and Cunene horse mackerel (*Trachurus trecae*) from Angolan waters were used during this workshop. The Namibian Cape horse mackerel specimens were collected during the routine annual horse mackerel surveys conducted by the research vessel Dr. Fridtjof Nansen in 1996 and 1997. The survey area covered the entire Namibian shelf between the Orange and Cunene Rivers. Following the recommendation made in the 2000 workshop, otoliths were stored dry in plastic vials, protected by cotton wool to prevent damage to the otoliths. A total of 298 specimens (ranging from 13 to 45 cm TL) were extracted during the surveys. A sub-sample of 230 otoliths from fish longer than 25 cm TL was selected, and the otoliths prepared before and during the workshop.

The South African specimens were collected between October 1999 and January 2000 on the south coast (between 24^{o} E and 26^{o} E) from a commercial mid-water trawler (the "Golovkov"). A total of 1117 otoliths were extracted aboard the vessel and stored in paper envelopes. A sub-sample of 109 specimens (5 specimens per 1cm size class where available) falling within the size range 24 to 48 cm total length (TL) were randomly selected and prepared (see section 4.2.) during the workshop.

The Cunene horse mackerel samples were collected during the 2000 and 2001 surveys conducted by the Dr. Fridtjof Nansen in Angolan waters. The otoliths were stored dry in paper envelopes. A total of 263 unprepared otoliths were available, of which 85 specimens falling within the size range 16 to 43 cm TL (approximately 3 individuals per 1 cm size class) were randomly selected and prepared for examination during the workshop.

4.3. Otolith preparation:

Following the recommendation of the 2000 workshop, all otoliths were prepared using the same method. The position of the nucleus was marked on the otolith surface using a pencil, and the otoliths were then burnt using a spirit burner until the colour had changed from white to brown. Each otolith was subsequently embedded in clear polyurethane resin, and then sliced using an "Isomet" low speed geological saw equipped with diamond coated blades. All otoliths were sliced in the transverse plane such that the nucleus was included in the slice. In the case of the Namibian specimens, four blades were used simultaneously, generating three parallel slices for each otolith. The South African and Angolan specimens were sliced using only two blades, yielding a single slice for each otolith. All slices were mounted on glass microscope slices using DPX microscope mounting medium. Preliminary observation of the Namibian specimens indicated that the amount of burning was generally insufficient to adequately enhance the contrast of the hyaline and opaque zones of the otoliths. The South African and Angolan otoliths were consequently subjected to more intense burning prior to being embedded and sliced. Completed otolith preparations were stored in slide trays, and labeled with only a reference number.

4.4. Viewing of otoliths:

For the purposes of group discussions, specimens were viewed with an Olympus SZ 60 stereo microscope equipped with both transmitted and incident light sources. A Sony digital colour video camera, interfaced to a personal computer, was fitted to the microscope. Live images were then viewed on the monitor using the "analySIS®" software available from Soft Imaging Systems GmbH. Where required, images were captured using the software for later editing and printing.

For the purposes of age estimation, all participants were provided with stereo microscopes equipped with both incident and transmitted illumination. Initially, several of the microscopes were found to be of inferior quality, particularly with respect to the illumination. This obstacle was overcome after the first reading session by the acquisition of suitable microscopes, resulting in all participants having access to microscopes and light sources of similar quality.

4.5. Reading (interpretation) of otoliths:

The objective of the age estimation exercise was to assign an age group representing a year class to each otolith. For this, the number of complete annual growth zones (AGZ's) had to be established by counting the number of annuli. Considering that the specimens being used were slices, rather than whole otoliths or sections that were used in the 2000 workshop, the participants had to develop a new set of criteria regulating annulus identification. These criteria were developed during the group discussions with Dr. Michael Kerstan. The approach was to identify alternating opaque and hyaline zones displaying maximum contrast. The burning procedure used during the otolith preparation process was aimed at enhancing the contrast between these zones. The burning results in the opaque zones (the white, calcium-rich bands apparent in untreated otoliths) remaining "light" (i.e. allowing transmission of light), whereas the "hyaline" zones (visible as "dark" bands in untreated otoliths), are visible as dark brown bands.

The participants established that two possible sets of criteria could be employed to identify AGZ's, depending on the structure and contrast of the otolith itself. Firstly, an annulus could be defined as a prominent dark band (i.e. hyaline zone) apparent before a more diffuse "light" zone (Figure A). In many cases, however, the hyaline zones are not well defined (i.e. limited contrast), but the following opaque zone (manifested as a "light" band) was very prominent (Figure A). Consequently, an alternate criterion would be to count the number of prominent light bands, representing the beginning of each AGZ. In many cases, annuli are not well defined along the sulcal axis of the slices (and this is particularly the case with the first annulus). In such cases, it is useful to extend examination to the lateral surface of the otolith, where surface structural features can be of great value in identifying annuli (Figure A).

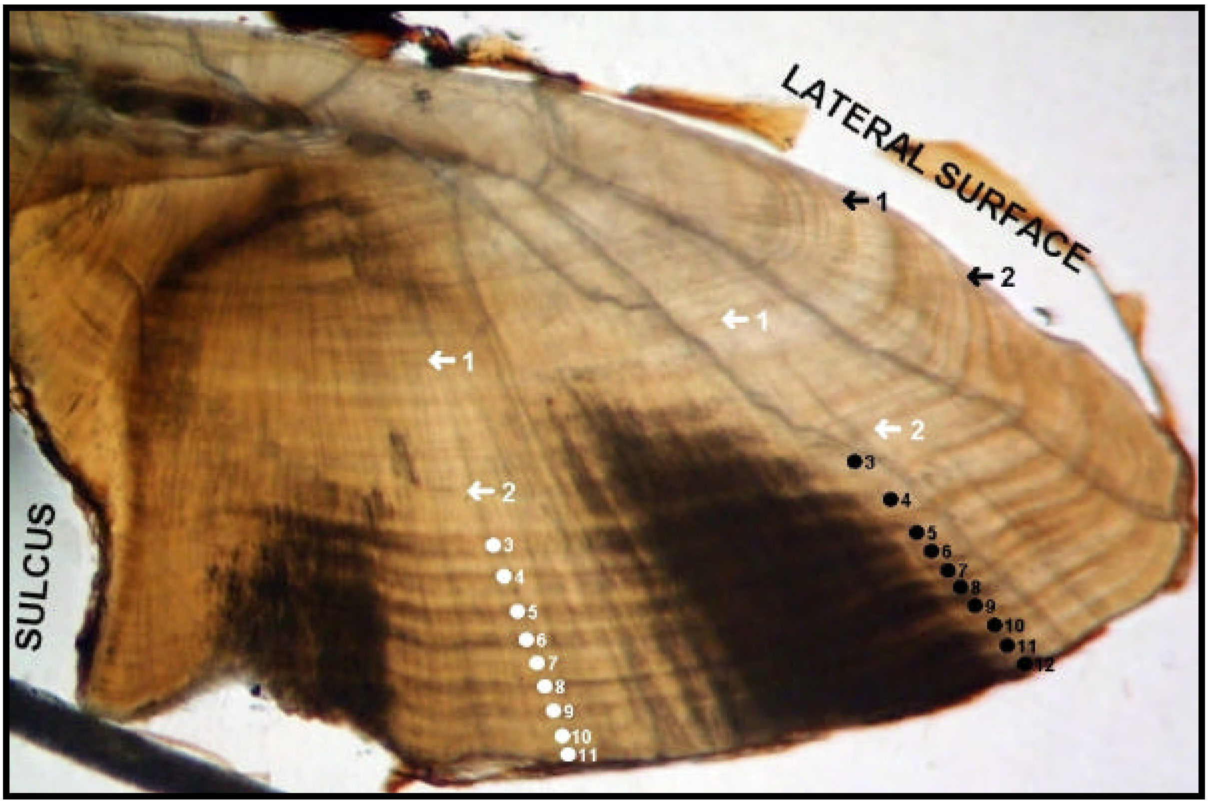

Figure A: Micrograph illustrating annual growth zone (AGZ) identification criteria. Identification criteria that can be applied are either prominent, dark hyaline zones at the outside edge of each AGZ (white dots), or broad, translucent zones forming the first part of each AGZ (black dots). Examination of the lateral surface assists in the identification of annuli (black arrows) that are not readily identifiable in the rest of the otolith (white arrows). Burnt and sliced otolith from a 45cm Cunene horse mackerel (*Trachurus trecae*) caught in August 2001. Eleven complete AGZs are visible, leading to the participants assigning a modal age of 11 to this fish. However, considering the marginal increment (black dot #12) in combination with the catch date, it is likely that this fish should be assigned to age group 12.

Once a count of the number of complete AGZ's had been obtained, the degree of completion of the marginal increment (MI) was subjectively assessed. The MI is that part of the otolith between the outside edge of the last complete AGZ and the edge of the otolith. The degree of completion of the MI was assessed relative to the width of the previous (complete) AGZ. Because it is generally very difficult to quantify the degree of completion of the MI without accurate measurements, the participants decided to employ a relatively coarse scale to assess the MI:

-	=	no MI (last AGZ complete)
+	=	AGZ about one third complete
++	=	AGZ about two thirds complete
+++	=	AGZ almost complete

The MI value, combined with the catch date of the individual, was then used to allocate the individual to an age group and year class according to the "winter-to-winter" year class convention in the Southern Hemisphere. This convention presumes that all individuals hatching between 1 July of a given year and 30 June of the following year belong to the same year class. Because most pelagic species in the Southern Hemisphere display peak spawning periods during summer, the winter-to-winter convention ensures that the year class definition will encompass all individuals from the same spawning season (Figure B).

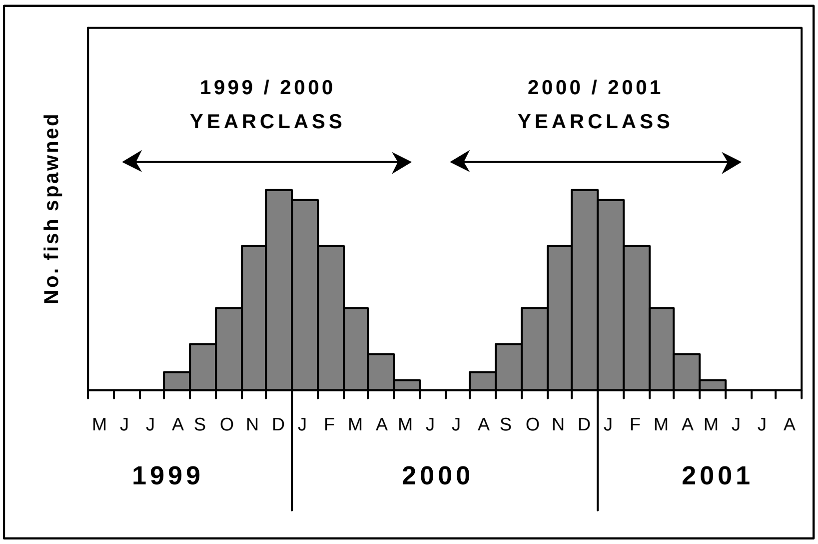

Figure B: The "winter-to-winter" year class convention adopted for Southern hemisphere species.

The rule applied during the age group and year class allocation procedure was that each reader had to subjectively assess whether the fish would have completed depositing the currently incomplete AGZ (i.e. the MI) before the next 1 July. If the MI would be complete by 1 July, the age group of the fish would increase by one year over the number of complete AGZ's that were counted on the otolith. Table A provides several examples to illustrate this. In cases where it was difficult to assess whether or not the MI would have been completed by 1 July, the participants adopted a precautionary approach and allocated the individual to the age group corresponding to the number of complete AGZ's counted on the otolith (see examples E, F and G in Table A).

Table A: Examples of age group (AGE) and year class assignments based on the number of complete annual growth zones (AGZ) and the width of the marginal increment (MI), relative to the catch date.

Catch Date	AGZ	MI	Age	Year Class
A. 2 May 2000	4	+	4	1995 / 1996
B. 14 July 2000	4	+	5	1994 / 1995
C. 12 January 1998	5	++	6	1991 / 1992
D. 29 March 1998	5	++	5	1993 / 1994
E. 2 January 2000	3	+	3	1996 / 1997
F. 2 January 2000	3	++	3	1996 / 1997
G. 2 January 2000	3	+++	4	1995 / 1996

For analyses of reader precision and bias, the data recorded by each reader were age group assignments for each individual, following the procedure described above.

4.6. Data analyses:

The data collected during this workshop comprised three replicate age group assignments for each otolith by each of the five participants. Because the three otolith sets reflected different geographical areas, as well as different species, each otolith set was treated independently. As discussed in section 1, one of the primary objective of this workshop was to assess the stability of the criteria each reader employed to interpret otolith structure. This was assessed in a number of different ways. Firstly, the number of the otoliths analyzed that were assigned the same age in all replicates (i.e. 100% agreement) as opposed to different ages in all replicates (0% agreement) were computed. Secondly, the magnitude of the differences between the three replicates recorded by each reader were quantified as the difference between the highest and lowest ages assigned to each otolith (i.e. replicate deviation) and plotted as frequency distributions. The final approach was to compute three different indices of the precision of the three replicate age assignments recorded by each reader for each otolith. The indices used were the percent agreement (% AGR), the average percent error (APE) statistic proposed by Beamish and McFarlane (1981), and the coefficient of variation (CV, equivalent to the standard deviation divided by the mean and expressed as percentage). % AGR was computed as the percentage of the replicate readings that were equivalent to the modal age calculated for each otolith. Although this index of precision is gradually falling out of favour (Campana *et al.*, 1995), percent agreement values were computed for comparative purposes. For the purposes of this workshop, percent agreement values above 70%, or CV's below 10% (corresponding to an APE below 7.2%, using the relationship between CV and APE provided by Campana, 2001) were considered to be acceptable levels of precision. Campana (2001) suggests that CV's of less than 7.6% (corresponding to APE's of less than 5.5 %) are the norm for most fish age estimation studies.

In the opening section of this report, it was emphasized that because otoliths from fish of known age are not currently available, bias has to be measured relative to some reference value, rather than the true age. Two potential candidates for a reference age are the rounded mean age, and the modal age. There is a fundamental difference in the philosophy underlying these two estimates of age. Given a series of age readings for a particular otolith (whether recorded by a single reader, or by several different readers), the modal age is computed as the most frequently occurring age estimate in the series. In other words, the modal age reflects the "most often agreed upon" age assignment. In contrast, the mean age is merely the average of the series of estimates. The participants felt that modal age is a more meaningful value to use as reference age, and this value was consequently adopted for all the bias analyses described below. In several otoliths, modal ages could not be computed because none of the replicate age estimates agreed. The data from these specimens were consequently excluded from further analyses.

Three different approaches were used to assess the levels of bias inherent in the age readings generated by the readers. Bias was assessed statistically using the non-parametric Wilcoxon matched pairs rank test. In practice, bias was computed using the work table for bias testing in the MS Excel worksheet "EFAN Age Comparisons Worksheet" provided by Eltink (2000). Depending on the absolute magnitude of the z statistic calculated during the test, this work table generates results that indicate either no bias ($|z| < 1.96$), a possibility of bias ($1.96 < |z| < 2.58$), or a certainty of bias ($|z| > 2.58$). For within-reader bias comparisons, the age estimates recorded by a given reader for a given replicate reading was compared to the modal age computed from the three readings for each otolith by the reader. Among-reader comparisons were computed for each reading by comparing the age estimates of each reader for a given replicate reading to the modal age computed from the five readings obtained by the five different readers during that reading. Overall among-reader comparisons were also conducted where the modal age computed from the three replicate readings by each reader was compared to the modal age computed from the fifteen age estimates recorded by all readers over all three replicate readings.

The two other approaches used to assess bias employed graphical representations of the data. The first, a within-reader comparison involved plotting the average residuals (± 1SE) of the age estimates recorded during each replicate reading. For this purpose, the difference between each age estimate and the modal age computed over the three readings was averaged over all otoliths to generate an average residual for each reading. Average residuals below zero for a given replicate indicate a tendency to under-estimate modal age, whereas average residuals above zero indicate a tendency to overestimate modal age. In addition, the age-bias plots recommended by Campana *et al.* (1995) and Eltink *et al.* (2000) were also generated for both within and among reader comparisons. The average (± 2 SE) of the age estimates obtained by a given reader corresponding to the modal ages computed over the three estimates recorded by the reader (within-reader age-bias plots), or the five estimates recorded by all readers during a given reading (among-reader age-bias plots), or the fifteen estimates recorded by all readers during all readings (overall among-reader age-bias plots) were plotted against the modal ages and compared to the 1:1 relationship that represents no bias.

5. RESULTS AND DISCUSSION

Five readers were involved in the otolith reading process during this workshop:

Reader 1 - Michael Kerstan (**MK**)
Reader 2 – Deon Durholtz (**DD**)
Reader 3 – Frances Dealie (**FD**)
Reader 4 – Vicky Herbert (**VH**)
Reader 5 – Henriette Lutuba (**HL**)

All 5 readers conducted 3 replicate readings on the otoliths from Cape horse mackerel (*Trachurus trachurus capensis*) from Namibia (otolith set **NAM**) and South Africa (otolith set **SA**), and Cunene horse mackerel (*Trachurus trecae*) from Angola (otolith set **ANG**). Note that in the discussion of the results of the bias analyses below, references to “younger” and “older” fish are relative to the measure of reference age employed during these analyses, namely modal age. As was discussed earlier in this report, modal age is purely an estimate of the most likely age of the fish by either individual readers (within-reader comparisons), or by the group as a whole (among-reader comparisons). It cannot be considered to be an absolute measure of the true age of the fish.

5.1. Cape horse mackerel (*Trachurus trachurus capensis*) - NAM

5.1.1. Preparation and Readability:

Of the 230 otoliths that were prepared, 52 (from individuals covering a size range of 28 to 44 cm total length, Figure 1) were considered to be satisfactory for age estimation purposes (a success rate of 23%). The reasons for this low success rate were:

- Many of the otoliths were insufficiently burnt, and displayed limited contrast between the opaque and hyaline zones.
- Carbon residue from the spirit burner often obscured the features in many of the specimens.
- Several of the otoliths were not sliced in the correct plane (i.e. through the nucleus), primarily because the marking of the nucleus with a pencil before preparing the otoliths was not done under a microscope. This resulted in the participants experiencing difficulties in identifying the annuli, because the appearance of these features differs depending on where the slice was made relative to the nucleus.
- The slices were of variable thickness. Slices that were too thin either cracked (obscuring the features of interest) or disintegrated. In several cases, the slices were too thick, making it very difficult for the readers to detect and distinguish the features used to estimate age.

Although the methodological shortcomings were primarily responsible for variable readability of the specimens, the nature of the otoliths also contributed to this problem. For example, many of the specimens displayed double rings (although this was not as prevalent as in the South African specimens, see section 5.2.1), often leading to difficulties with annulus recognition along the sulcus axis. In several of the otoliths displaying double rings, annulus identification could be confirmed by examining structural features on the dorsal surface of the otolith (see Figure A).

A further factor contributing to the variability in the results was that several of the readers (namely readers 1, 2 and 4) were using microscopes and illumination of inferior quality during the first readings of this otolith set. This obstacle was overcome after the first reading. Several of the participants experienced problems with the assessment of the marginal increment, and subsequent age group and year class assignment of the specimens relative to the catch dates. This topic had to be revisited on several occasions. The procedure to be followed has been outlined in detail in section 4.4.

5.1.2. Precision:

The precision with which the participants read the otolith set NAM was generally acceptable within each reader, but not among the group as a whole. Less than half of the otoliths were assigned to the same age group in all three readings by each reader (i.e. 100% stability, Table 1), with readers 1 and 5 displaying the highest stability. Most of the remaining otoliths were assigned to the same age group in at least two of the readings, with only between 2 and 5 otoliths being assigned to a different age group with each reading (i.e. 0% stability, Table 1). Age group assignments for the same otolith generally varied by between 1 and 2 years (Figure 2a), and occasionally by 3 or 4 years in the cases of readers 3 and 4. Treating the group as a whole (i.e. considering all 15 estimates recorded by the readers), age group assignments for a given otolith often deviated by as much as 5 years, with the majority differing by about 2 years. These observations are reflected in the three indices of precision. Percent agreement values ranged between 70 and 80% for all readers (corresponding to CV's of between 6.9 and 10.1%), with readers 1 and 5 displaying the highest levels of precision. These values indicate that each reader is employing relatively stable interpretation criteria, sufficient to generate consistent age length keys. This conclusion does not apply to the group as a whole (which has relevance if shared stocks are being assessed, and age estimates obtained from different readers are to be employed). Overall levels of precision were only a 56 % AGR, corresponding to an APE of about 12%, and a CV of 14% (Table 1). These results indicate that different interpretation criteria are being employed by the readers, with the implication that age group assignments for the same otolith would differ among the readers.

5.1.3. Bias:

Within-reader comparisons:

The results of the Wilcoxon matched pairs rank tests indicate that the readers were generally unbiased in their successive replicate readings (Table 2, Figure 3a). The only exception was a possibility of bias in the third reading by reader 2, where the age estimates tended to be lower than the modal age (11 underestimates versus 2 overestimates, Table 2, and see Figure 3a). These underestimates were apparent mainly at intermediate modal ages (Figure 4a). These observations support the results of the precision analyses, in that the interpretation criteria being employed by each reader remained relatively stable over the successive readings of each otolith. An interesting observation is that neither the Wilcoxon tests nor the residual plot of the results of the first reading conducted by reader 5 detect the marked tendency by this reader to overestimate the age of younger fish, and underestimate the age of older fish (see Figure 4a). It should be noted that reader 5 corrected this tendency in subsequent readings (Figure 4a).

Among-reader comparisons:

With the exception of reader 1 where a tendency to overestimate age relative to the group, albeit almost negligible, indicated a possibility of bias (Table 3), the Wilcoxon tests indicated that the readers were unbiased during the first reading. However, the age-bias plots (Figure 5a) reveal that reader 3 showed a marked tendency to underestimate the age of older fish, while readers 4 and 5 both tended to overestimate the age of younger fish, and underestimate the age of older fish (relative to the group). These trends were still apparent in the second reading (Figure 5b), where readers 1 and 2 conformed closely to the interpretation of the group as a whole, while reader 3's increased tendency to underestimate the age of older fish suggests the possibility of bias (Table 3). Readers 4 and 5, while appearing to correct their interpretation of younger fish, still underestimated the age of older fish relative to the group. During the third reading, most of the readers were beginning to conform more closely to the group interpretation, none of the Wilcoxon tests indicating bias (Table 4). The age-bias plots (Figure 5c) do reveal, however, that reader 2 was increasingly tending to underestimate modal age, reader 3's tendency to underestimate the age of older fish was still apparent, and while readers 4 and 5 appeared to have corrected their interpretation of older fish, these readers were again overestimating the age of younger fish.

An overall representation of the results (Figure 5d) clearly summarizes these trends. Readers 1 and 2 best reflect the group estimation of fish age, whereas readers 4 and 5 tend to over- and underestimate the age of young and old fish respectively. Reader 3, while conforming to the group's interpretation of younger fish, shows a marked tendency to underestimate the age of older fish.

5.2. Cape horse mackerel (*Trachurus trachurus capensis*) - SA

5.2.1. Preparation and Readability:

The success rate of the preparation of the South African otolith set was considerably higher than that of the Namibian set. Ninety-one specimens (covering a size range of 25 to 48 cm total length, Figure 1) of the original 109 were considered satisfactory, indicating a preparation success rate of 83.5%. Although problems with the technical preparation of the otoliths resulted in many of the specimens having to be discarded (resulting from different personnel being involved in their preparation), experience gained from the preparation of the Namibian otolith set improved the success rate of the South African otolith preparation through improved burning and slicing procedures. A major difference in the readability of the South African otoliths, compared to the Namibian and Angolan otoliths was apparent. A considerably higher proportion of the SA otolith set displayed more double and/or false rings than did either the NAM or ANG otolith sets, contributing substantially to age estimation variability. This difference could be largely attributed to biological factors, rather than technical/preparation and reader deficiencies, in that the multitude of irregularly spaced false rings apparent in the SA otolith set presumably reflects the more variable environmental regimes available to the fish in South African waters.

5.2.2. Precision:

The complicated otolith structure and a consequent difficulties experienced with the interpretation of the SA otolith set are clearly manifested in the precision of the age estimates obtained from these otoliths. Relative to the NAM otolith set, a higher proportion of the SA otoliths were considered to be unreadable by several of the participants (Table 1). In terms of stability, substantially lower numbers of SA otoliths were assigned to the same age group in all three readings (i.e. 100% stability, Table 1) in comparison to the NAM set, with readers 1 and 3 again displaying the highest levels of stability. The number of readings displaying 0% stability were correspondingly higher, with up to 25 of the 91 otoliths being assigned to different age groups in each successive reading (reader 3). The magnitude of the deviations between the 3 replicate readings were also much higher than in the NAM otolith set (Figure 2b). Age assignments for a given otolith by a given reader differed by as much as 6 years in some cases, although the majority differed by 1 to 2 years. Considering all participants as a group, none of the otoliths were assigned the same age by all readers in all readings. In most cases, age assignments differed by 3 to 4 years, and in some cases by up to 10 years (Figure 2b, "OVERALL"). The indices of precision were correspondingly inferior to the NAM otolith set. Only readers 1 and 5 displayed satisfactory levels of precision (about 73% agreement, and CV's of 10 and 8.8% respectively), with reader 5 being slightly more precise than reader 1 (Table 1). The age estimates of the other three readers showed high levels of imprecision, with CV's of between 15 and 19%, and percent agreement values below 60%. As a group, the overall levels of precision were low (42% agreement and CV of almost 25%). The participants, acting as a group would consequently not be able to generate consistent age length keys for horse mackerel from South African waters. Only readers 1 and 5, acting as individuals, would be able to produce satisfactory ALK's for the South African specimens.

5.2.3. Bias:

Within-reader comparisons:

In spite of the low levels of precision with which the participants interpreted the SA otolith set, the results of the Wilcoxon matched pairs rank tests indicated that the readers were generally unbiased in their successive readings (Table 2). As with the NAM otolith set, an exception was reader 2, where the tendency to overestimate modal age during the third reading (5 underestimates versus 10 overestimates, Table 2, Figure 3b) at intermediate and high modal ages (Figure 4b) indicated a possibility of bias. Reader 3 also displayed a possibility of bias during the first reading (Table 2, Figure 3b), where the age estimates tended to overestimate modal age, particularly at low and high modal ages (Figure 4b).

The result that the readers were generally unbiased in their successive readings is surprising, considering the low levels of precision discussed above (section 5.2.2). It should be noted, however, that the capacity of the Wilcoxon test to detect trends in bias is limited (Campana *et al*., 1995) and this is particularly apparent in the results of readers 3 and 4. Both of these readers displayed a marked tendency to increasingly underestimate modal age in successive reading (Figure 3b), suggesting that the interpretation criteria employed by these readers was undergoing a directional change between successive readings.

Among-reader comparisons:

The among-reader comparisons clearly illustrate that the readers employed different interpretation criteria to estimate the age of the SA otolith set, particularly with regard to the identification of annuli as opposed to false rings. The Wilcoxon tests indicated either a possibility or a certainty of bias in the successive readings of almost all the participants (Table 3). Most of the readers tended to overestimate the modal age of many of the otoliths during all readings, only reader 1 tending to age fish as younger than the group as a whole. This could be attributable to reader 1's prior experience with South African specimens, particularly with regard to the recognition of false and/or double rings as such. The tendency of readers 4 and 5 to respectively over- and underestimate the age of young and old fish in the NAM otolith set is apparent in this otolith set as well (Figure 6). Readers 1 and 2 again conformed most closely to the group interpretation, while the estimates of reader 5 were the most consistent (least variable) of all the readers. Successive readings of the otoliths did not appreciably alter these trends, and the overall age-bias plots (Figure 6d) are therefore a representative reflection of the bias inherent in the reading of the SA otolith set. These plots clearly illustrate the typical bias patterns exhibited by readers 4 and 5 (i.e. over- and underestimation of young and old fish respectively). Readers 2 and 3 generally overestimated fish age at all modal ages (indicating that these readers were identifying larger proportions of false rings as annuli), whereas reader 1 typically aged fish as younger than the rest of the readers. The relatively high level of consistency in the readings of reader 5 are clearly manifested in the low variability associated with the age estimates recorded by this reader.

5.3. Cunene horse mackerel (*Trachurus trecae*) - ANG

5.3.1. Preparation and Readability:

Of the 85 Cunene horse mackerel otoliths prepared during the workshop, 54 were considered to be satisfactory for age reading (size range 15 to 46 cm total length, Figure 1), reflecting an intermediate success rate of 63.5%. Although many of the slices were either too irregular, too thin or sliced in the incorrect plane (due to a different technical assistant preparing these otoliths), the burning procedure was generally conducted satisfactorily, hence the intermediate success rate. The readability of the ANG otoliths was generally very similar to that of the NAM specimens, although several of the otoliths displayed features similar to those observed in the SA otoliths. Although the results of the 2000 workshop suggested that otoliths may not be an option for the age estimation of Cunene horse mackerel, the results of this workshop indicated otherwise. The burning and slicing procedure employed during this workshop generated specimens of generally satisfactory readability, indicating that it is not necessary to develop a new age estimation method for this species.

5.3.2. Precision:

The precision of the age estimates obtained from this otolith set were comparable to those from the NAM set, and in several cases, slightly better. Similar proportions of otoliths were assigned to the same age group in all replicate readings (Table 1), with readers 1 and 5 again being the most stable in their interpretation. The magnitude of the deviations in the age assignments over the three readings by each reader (Figure 2c) were almost identical to those observed in the NAM otolith set, with most age assignments for a given otolith by a given reader deviating by only 1 to 2 years. In some cases, age assignments differed by up to 4 years, but these were few in number. The group as a whole generally differed in their age assignments by about 2 years, with a few specimens displaying differences of up to 7 years (Figure 2c, “OVERALL”). With the exception of reader 3, the levels of precision realized in the age readings from the Cunene horse mackerel otoliths were the highest of all three otolith sets, with CV’s below 10% and percent agreement values in excess of 70% (Table 1). Readers 1 and 5 again displayed the highest levels of precision, and reader 3 the lowest. The imprecision of reader 3’s estimates contributed directly to the relatively low precision of the group as a whole (56.8% agreement, 16.5% CV). The results indicate that with the exception of reader 3, the criteria employed by each participant to estimate the age of Cunene horse mackerel are sufficiently stable to permit the generation of consistent age length keys for this species.

5.3.3. Bias:

Within-reader comparisons:

Results of the Wilcoxon tests (Table 2, and see Figure 3c) indicated a certainty of bias in the second reading by reader 1 (overestimation) , and in the first readings of readers 3 (overestimation) and 5 (underestimation). A possibility of bias was also indicated for the third reading of reader 3 (underestimation). Reference to the age bias plots illustrated in Figure 4c reveals that the deviations of the age estimates from the modal ages in these cases is almost negligible. It is their cumulative effect that leads to the significant results of the Wilcoxon test. In view of this observation (and those discussed with regard to the results from the other two otolith sets), it is felt that not much weight should be attributed to the Wilcoxon test results. Rather, the trends (or lack thereof) apparent in the residual (Figure 3) and age-bias plots (Figure 4) should be considered as realistic representations of the stability of the interpretation criteria employed by each participant over successive readings. For example, although the Wilcoxon tests detected the strong tendency by reader 3 to increasingly underestimate modal age of the ANG otoliths with successive readings (a trend also apparent in this reader's interpretation of the SA otolith set), they failed to detect the same tendency apparent in the readings of reader 4 (Figure 3c).

Among-reader comparisons:

The Wilcoxon test results indicated a certainty of bias in the first readings of readers 3 and 5 (Table 3), reader 3 tending to overestimate age (particularly in younger fish, Figure 7a), whereas reader 5 tended to underestimate age in all but the youngest fish. Readers 1, 2 and 4 generally conformed to the group's interpretation, although a slight tendency to overestimate age in younger fish and underestimate the age of older fish is apparent (Figure 7a). Similar trends were evident in the second reading (Figure 7b), although the tendency by readers 3 and 4 to underestimate the age of older fish was more pronounced (significantly so in the case of reader 4, Table 3), and these trends were carried through to the third reading as well (Figure 7c). These results contributed to the general, overall trend by readers 3 and 4 (and to a much lesser extent by readers 2 and 5) to underestimate the age of older fish (Figure 7d). Reader 1 consistently conformed to the group's interpretation with regard to modal age, and generally showed the least variability in the age estimates.

6. CONCLUSIONS

- The burning and slicing method of preparing horse mackerel otoliths generates specimens of suitable quality for age estimation purposes, provided technical shortcomings and inadequacies are overcome.
- With the exception of the South African specimens, the stability of the interpretation criteria employed by each of the participants to estimate age from otoliths was generally acceptable, although further improvement would be preferable. The directional changes in interpretation criteria with successive readings by several of the participants need to be explored.
- The problem of the relatively poor readability of otoliths from South African waters needs to be overcome. Examination of both untreated and burnt and sliced otoliths from the same fish may resolve the problems of false and double rings apparent in these specimens.
- The interpretation criteria employed by the participants do, however, vary substantially among readers, particularly with regard to younger and older fish (which are the important age groups for VPA assessments). The participants would consequently generate very different age length keys from the same otolith samples.
- The use of data collected during this workshop to produce age length keys is not supported by the results of the analyses. Although relatively consistent age length keys may be generated by several of the participants (particularly readers 1 and 5), they would be too variable among the readers to establish which would be the most representative of the stock(s).

7. RECOMMENDATIONS

7.1. General:

- Several of the readers require further training. Interpretation criteria employed by these readers are currently too variable for consistent age estimation.
- The interpretation criteria employed by all participants require further standardization. Age length keys produced by the different readers would not be comparable.
- Otolith reference collections should be produced for the region. A collection of this nature would be of great value to researchers involved in horse mackerel age estimation, facilitating the development and standardization of otolith interpretation criteria in the region (see section 7.3).
- The issue of personnel continuity is critically important, and should be addressed. Trained readers should be retained as readers for as long as possible. If a personnel change has to be made, there should be an over-lap period of suitable duration during which the incumbent(s) are trained and their interpretation criteria are calibrated against those of the previous reader. Data continuity has to be maintained.
- Where more than one reader is employed in an institution, the readers should inter-calibrate each other (i.e. replicate readings of the same otoliths should periodically be conducted by all readers to check for potential bias).
- Otolith preparation as well as equipment handling and maintenance should be the responsibility of one individual to maintain standards. Otolith preparation and resulting specimens should be of the highest quality possible since the reader(s) and resulting data depend entirely on the quality of the specimens.
- Trained technicians capable of generating high quality otolith preparations are extremely valuable personnel, and should be retained within the age estimation program at all costs. If a personnel change has to be effected, the incumbent should be carefully vetted and well trained.

7.2. Technical:

7.2.1. Otolith storage and handling

- The use of paper envelopes to store otoliths is not recommended because the otoliths are easily damaged. Glass or plastic vials with cotton wool at the bottom to protect the otoliths should rather be employed.
- Light forceps should be used when handling otoliths, and great care should be taken when extracting and cleaning otoliths at sea.

7.2.2. Otolith burning

- The position of the nucleus in the otoliths should be determined by microscopic examination, and the position marked on the otolith surface using a pencil, prior to burning.
- Spirit burners should not be used to burn otoliths because these burners often leave a carbon residue on the otoliths, obscuring features of interest. A residue-free technique should be used, preferably an oven, although gas burners are also adequate.
- Otoliths should be burnt at a temperature of about 350^{o}C until grayish-white in colour.
- If an oven is used, a graded series of burning times versus otolith size should be used to establish the optimum burning time.

- Small metal trays with numbered receptacles would permit several hundred otoliths to be burnt simultaneously, increasing efficiency.
- If a gas burner is being used, the otolith should be heated slowly. Rapid heating tends to damage the otoliths.
- The results of the burning should always be assessed using a microscope.

7.2.3. Otolith slicing

- A good otolith saw with a sledge allowing forward and backward movement, as well as lateral movement should be used. The gear ratio of the sledge should be very low, ensuring that the sledge moves forwards very slowly, exerting minimal pressure on the saw blades.
- Diamond-coated blades of good quality should always be used, and should be very well maintained. Care should be taken when slicing to avoid damage to the blades.
- The rotation speed of the blades should be high, ensuring that the slices are polished as they are cut.
- Slices have to be cut in the correct plane, including the nucleus in the slice. Further, all slices should be of the same thickness, with minimal damage to the specimens.

7.2.4. Microscopes

- These should be of the best quality possible, and be well maintained (preferably by the reader(s).
- Good illumination is imperative, and should allow for both transmitted and reflected lighting.
- Once suitable microscopes and illumination have been obtained, the same equipment (or at least the same models) should be used for as long as possible to maintain constancy.

7.2.5. Otolith reading (tips and hints)

- For age estimation of South African Cape horse mackerel, it is recommended that both otoliths from each fish sampled should be available for examination. One otolith should be burnt and sliced, while the other remains untreated. Examination of both specimens may facilitate the identification of annuli versus false or double rings. This approach should also be adopted for the Namibian and Angolan samples.
- When conducting an age estimation exercise, the nature of the age estimate should be established before beginning (e.g. precise age versus age group assignment), and the approach employed to arrive at the age estimate should be clearly stated and rigidly adhered to.
- In any age estimation program, it is recommended that otoliths from the largest (oldest) fish are analyzed first. This approach assists in establishing interpretation criteria.
- Whole otoliths as well as the broken and sliced preparations should be available to the reader(s) to assist in otolith interpretation. In other words, both sagittal otoliths should be extracted from each fish, one of which is burnt and sliced while the other is left untreated.
- When observing an otolith, it is useful to start at low magnification, because the features of interest are not obscured by fine detail. Magnification can then be increased where required, for example when looking at the margin of the otolith.

- When identifying annual growth zones (AGZ's), it can be assumed that the first and second AGZ's are generally wide, the third AGZ is usually of intermediate width, while the fourth and subsequent AGZ's are progressively narrower.
- The depth of the sulcus usually provides an indication of the age of the fish, with older fish generally displaying a relatively deeper sulcus.

7.3. Follow-up workshop:

In view of the results of this workshop, all participants agreed that a follow-up workshop is required. Two options were discussed:

- a workshop aimed at improving at further improving the precision of age reading (i.e. further stabilizing interpretation criteria), as well as starting to address the issue of accuracy.
- a workshop aimed at generating a reference collection for the region.

All participants supported the second option, and it was agreed to convene a workshop in late 2002 / early 2003 directed at producing a reference collection of horse mackerel otoliths for the Benguela region. This reference collection should:

- comprise a set of otoliths from which at least an 80% agreement in the age readings obtained by all readers from each otolith is realized.
- contain a range of otolith types (i.e. thick, thin etc.)
- represent a large sample size from as broad a size range of fish as possible, capable of generating a representative length-at-age distribution that can then be used for indirect validation (MULTIFAN could be used for this purpose).
- ideally be a sub-sample from a survey where many fish length data are available.
- comprise otolith preparations that are of superior quality, which have been prepared prior to the workshop.

Once the reference collection has been selected, the otoliths should be photographed and archived. The photographs could be distributed to researchers throughout the region for the purpose of calibrating and periodically checking reader otolith interpretation criteria, thereby contributing to standardizing horse mackerel age estimation in the Benguela region.

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10. TABLES

Table 1: Precision of the age estimates. The number of otoliths not read, the number of otoliths where all 3 readings were identical (100% Stability), the number of otoliths where all 3 readings differed from each other (0% Stability) are shown for each reader. Percent agreement (% AGR), average percent error (APE) and coefficient of variation (CV) values are averages computed over all otoliths for each reader, or all readers combined (Overall).

SET	READER	NOT READ	STABILITY		PRECISION		
			100%	0%	%AGR	APE	CV
NAM (n = 52)	1 – MK	0	24	2	79.49	5.26	6.94
	2 – DD	1	15	3	72.55	7.26	9.58
	3 – FD	0	19	5	72.44	7.62	10.13
	4 – VH	0	16	5	70.51	7.50	10.02
	5 - HL	0	21	2	77.56	6.18	8.15
	Overall				56.12	11.68	14.36
SA (n = 91)	1 – MK	0	36	10	72.53	7.62	10.08
	2 – DD	3	18	23	56.06	11.70	15.72
	3 – FD	1	9	25	51.48	13.94	18.80
	4 – VH	5	19	19	59.22	11.16	14.95
	5 - HL	3	31	6	73.86	6.66	8.81
	Overall				42.01	20.01	24.86
ANG (n = 54)	1 – MK	0	39	2	88.27	3.81	5.08
	2 – DD	0	21	6	72.22	7.29	9.68
	3 – FD	0	12	8	64.20	10.10	13.47
	4 – VH	1	19	2	76.10	7.04	9.23
	5 - HL	0	26	0	82.72	5.61	7.29
	Overall				56.82	12.99	16.54

Table 2: Within-reader bias comparisons. Results of the Wilcoxon matched pairs rank tests. Results from the three otolith sets (**NAM**, **SA** and **ANG**) are shown for each reader and each replicate. The number of underestimates (**-**) and overestimates (**+**) of modal age are indicated, as are the ***z*** statistic and resultant bias (***** = possible bias, ****** = certain bias).

SET	READER	1st READING			2nd READING			3rd READING		
		+	-	*z*	+	-	*z*	+	-	*z*
NAM	1 – MK	2	5	-1.0142	5	1	-1.4676	5	8	-0.9085
	2 – DD	6	5	-0.4890	1	8	-1.5993	11	2	-2.2713 *
	3 – FD	6	6	0.0000	6	4	-0.2548	4	2	-0.4193
	4 – VH	5	2	-1.0142	7	11	-0.6533	4	2	-0.3145
	5 - HL	11	6	-1.0888	1	3	-0.9129	5	3	-0.6301
SA	1 – MK	9	11	-0.6907	1	3	-1.0954	11	10	-0.5214
	2 – DD	11	8	-0.4024	9	4	-1.5025	5	10	-1.9879 *
	3 – FD	7	15	-2.2564 *	7	8	-0.2556	13	6	-1.3884
	4 – VH	6	14	-1.7920	3	4	-0.5916	14	6	-1.4560
	5 - HL	9	15	-1.5000	12	4	-1.5513	5	6	-0.4890
ANG	1 – MK	0	1	-1.3416	0	9	-2.6656 **	2	0	-1.3416
	2 – DD	7	4	-0.8002	5	5	-0.2548	3	3	0.0000
	3 – FD	3	12	-2.6410 **	4	7	-0.8002	7	1	-1.9604 *
	4 – VH	5	13	-1.8727	4	2	-0.7338	6	2	-1.4003
	5 - HL	15	2	-2.7693 **	2	7	-1.4809	1	1	0.0000

Table 3: Among-reader bias comparisons. Results of the Wilcoxon matched pairs rank tests. Results from the three otolith sets (**NAM**, **SA** and **ANG**) are shown for each replicate reading by each reader, and for all readings by each reader (Overall). The number of underestimates (**-**) and overestimates (**+**) of modal age are indicated, as are the ***z*** statistic and resultant bias (***** = possible bias, ******= certain bias).

SET	READING	READER 1 - MK			READER 2 - DD			READER 3 - FD			READER 4 - VH			READER 5 - HL		
		+	-	z	+	-	z	+	-	z	+	-	z	+	-	z
NAM	1st	8	15	-2.0074 *	7	5	-0.7060	10	13	-0.0912	7	12	-0.5231	16	10	-0.8000
	2nd	9	7	-0.0776	7	9	-0.1293	16	7	-2.0682 *	9	20	-1.2217	12	8	-0.7840
	3rd	5	12	-1.8462	15	7	-1.6070	10	8	-0.9363	5	12	-1.3255	12	9	-0.3650
	Overall	9	10	-0.9256	11	6	-0.9468	11	6	-1.4912	5	10	-0.7951	13	10	-0.8516
SA	1st	27	8	-2.4323 *	15	20	-0.8599	10	38	-3.5334 **	13	31	-2.0948 **	12	37	-4.1679 **
	2nd	19	8	-1.9941 *	12	26	-2.7482 **	7	42	-4.4067 **	15	24	-1.0885	12	38	-3.6248 **
	3rd	25	9	-3.0346 **	10	31	-3.4469 **	12	31	-2.5478 *	26	15	-2.0928 *	8	38	-4.2172 **
	Overall	31	10	-2.5658 *	12	25	-2.9041 **	10	31	-3.5700 **	23	24	-0.3280	10	37	-3.9313 **
ANG	1st	7	2	-1.7178	8	16	-1.7143	7	23	-2.6842 **	14	12	-0.6223	14	2	-2.7923 **
	2nd	7	5	-0.2353	10	14	-1.0571	11	18	-0.3568	17	3	-2.5386 *	11	11	0.0000
	3rd	11	8	-0.3823	6	17	-1.8553	8	15	-1.0493	12	7	-1.5091	5	12	-1.1598
	Overall	8	2	-1.6818	7	16	-1.9313	9	19	-1.0475	14	8	-1.7044	8	10	-0.0218

11. FIGURES

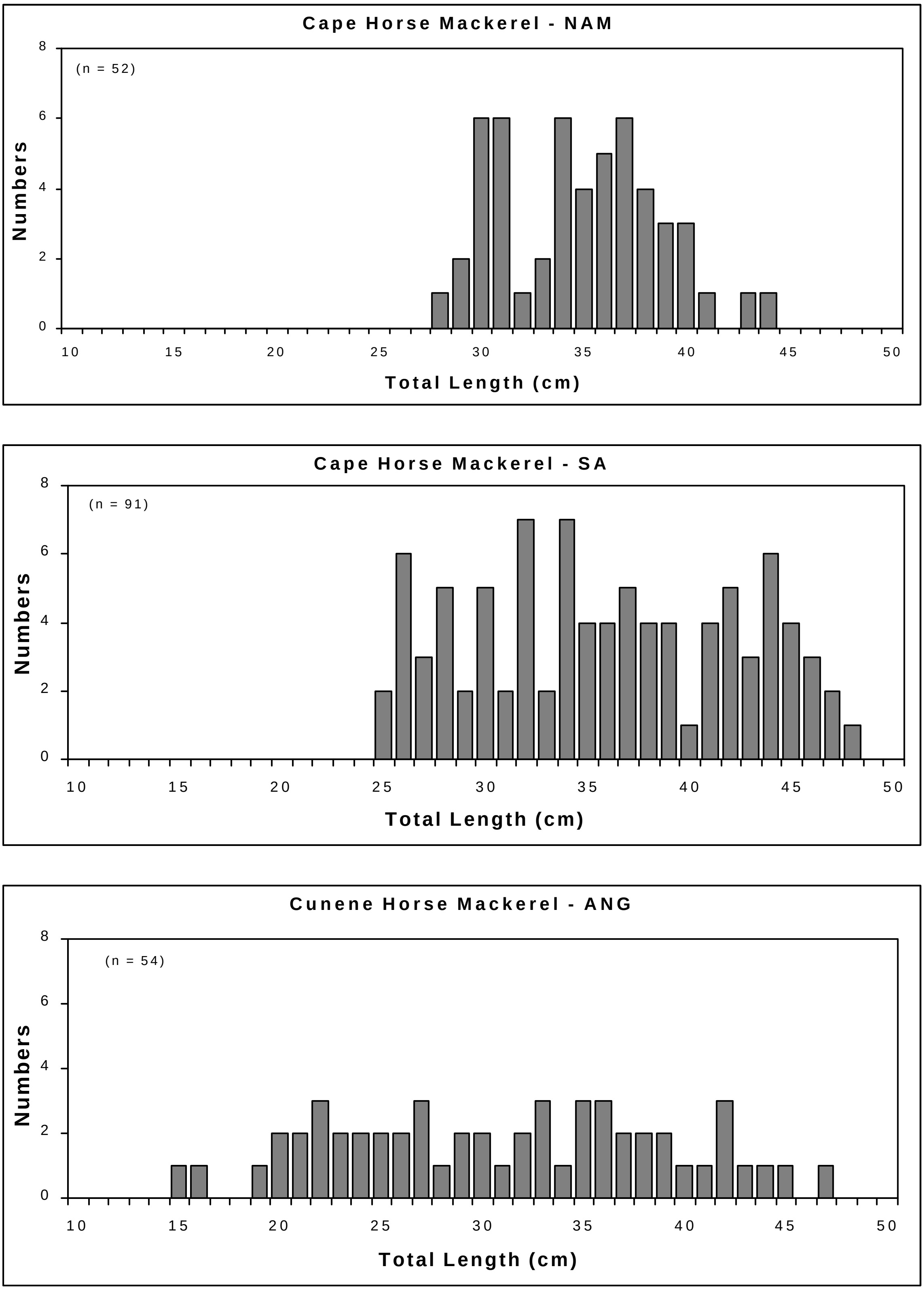

Figure 1: Length-frequency distribution of the horse mackerel from which the otoliths used in this workshop were extracted.

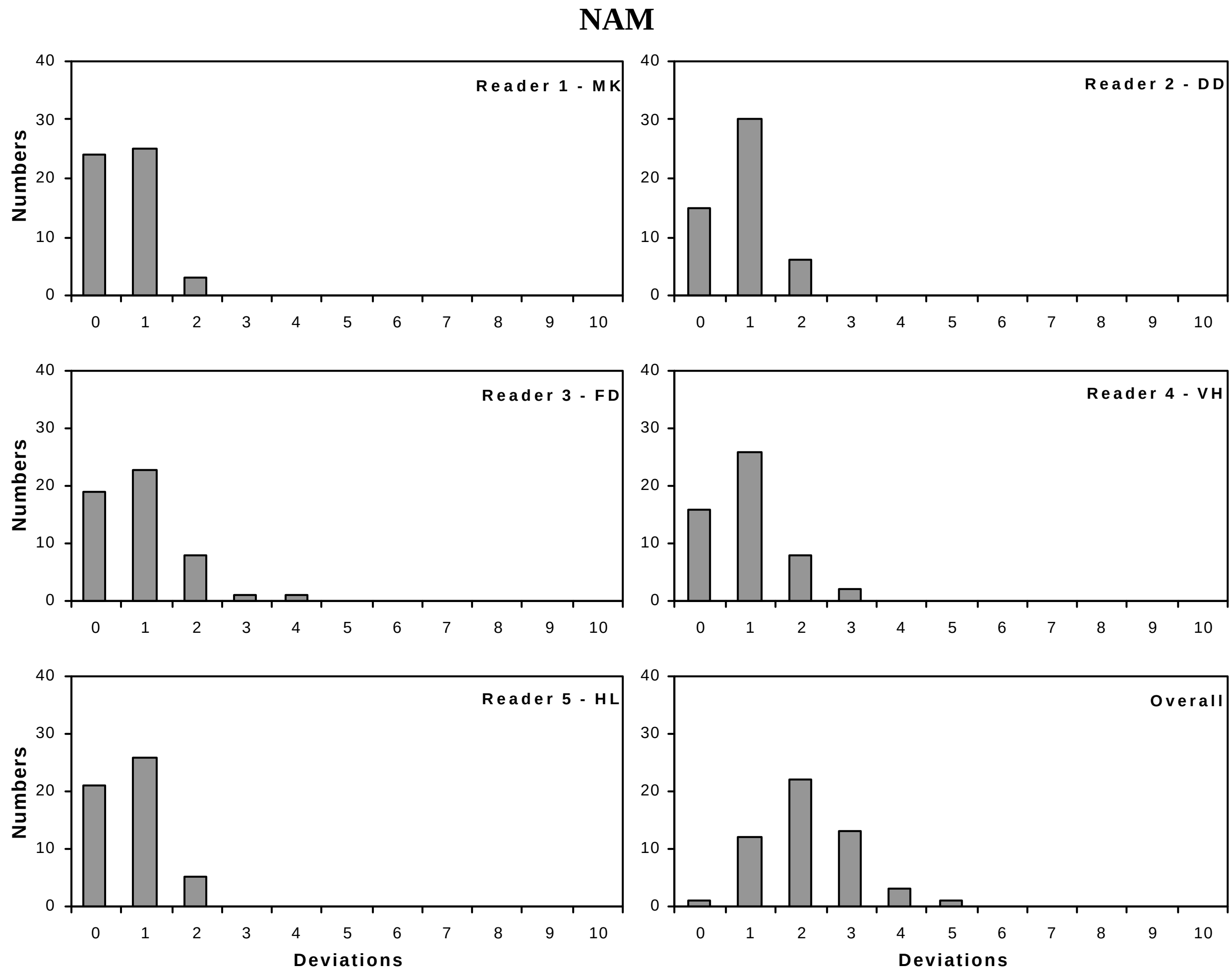

Figure 2a: Replicate deviations – **NAM**. Frequency distributions of the differences between the maximum and minimum age estimates recorded for each otolith by each reader.

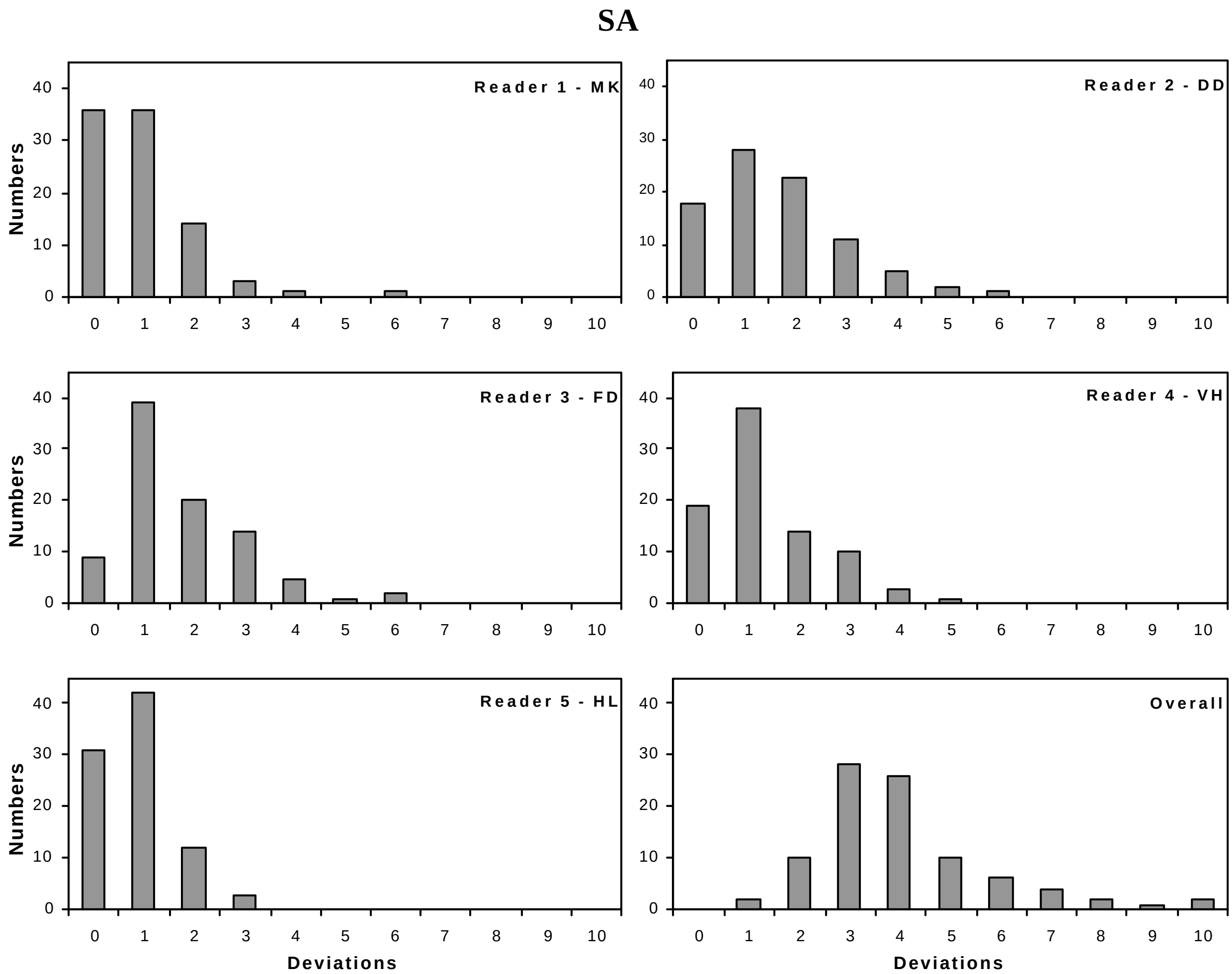

Figure 2b: Replicate deviations – **SA**. Frequency distributions of the differences between the maximum and minimum age estimates recorded for each otolith by each reader.

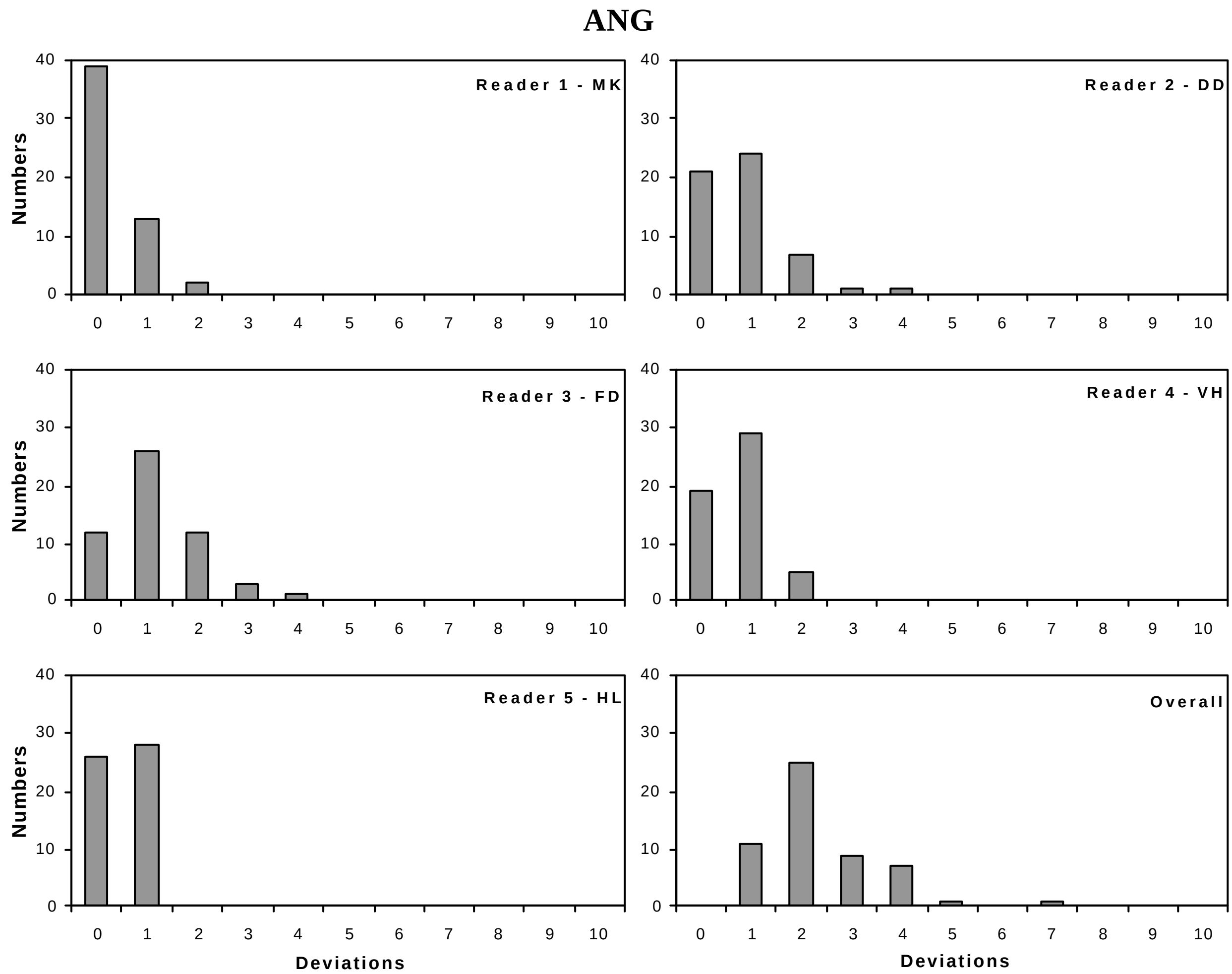

Figure 2c: Replicate deviations – **ANG**. Frequency distributions of the differences between the maximum and minimum age estimates recorded for each otolith by each reader.

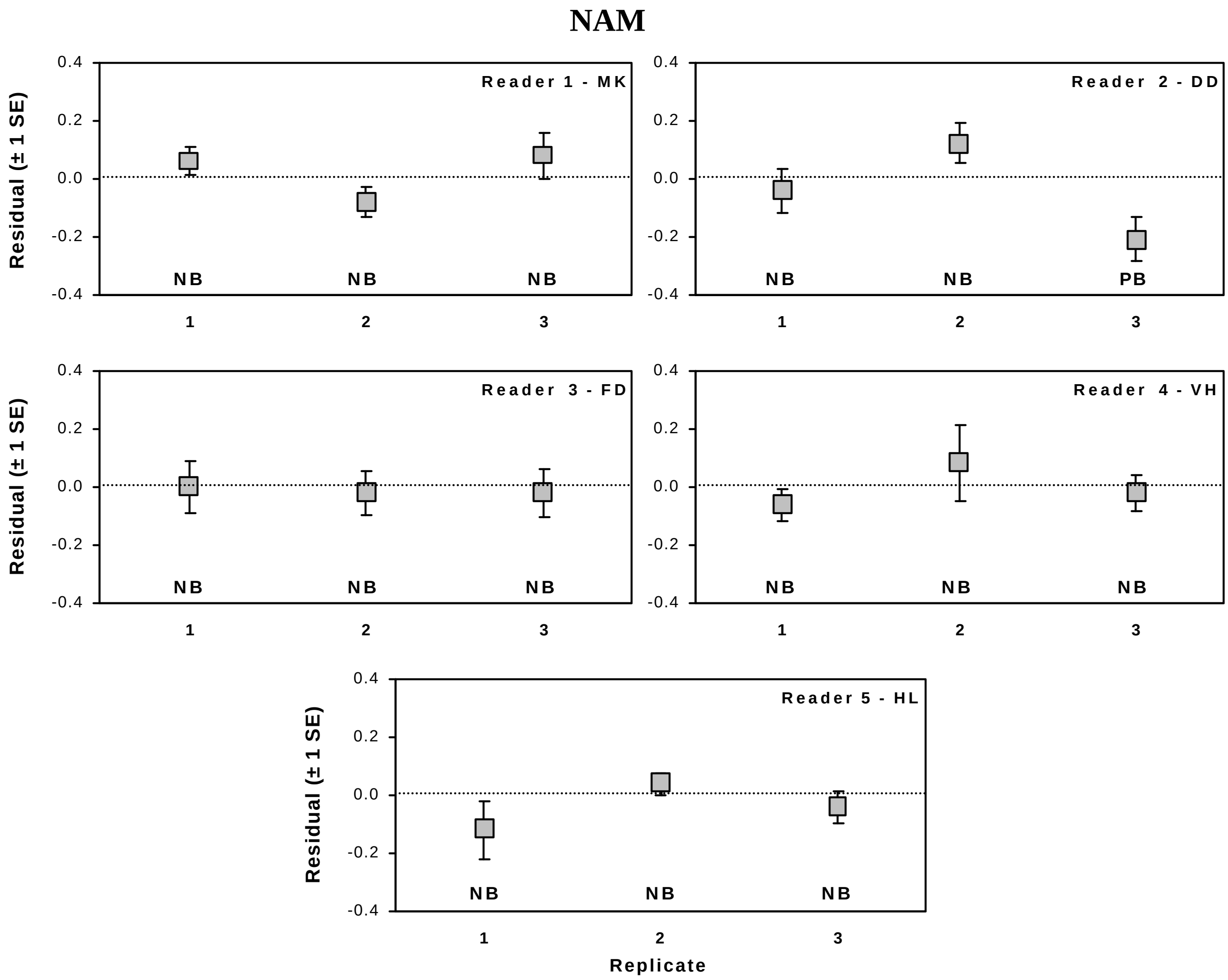

Figure 3a: Within-reader bias comparisons. Residual plots – **NAM**. The average residuals (± 1 SE) are plotted for each of the three replicate readings by each reader. Each residual represents the average of the deviations of the age estimates from the modal age of each otolith. The results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated as no bias (NB), possible bias (PB) or certain bias (CB) for each replicate.

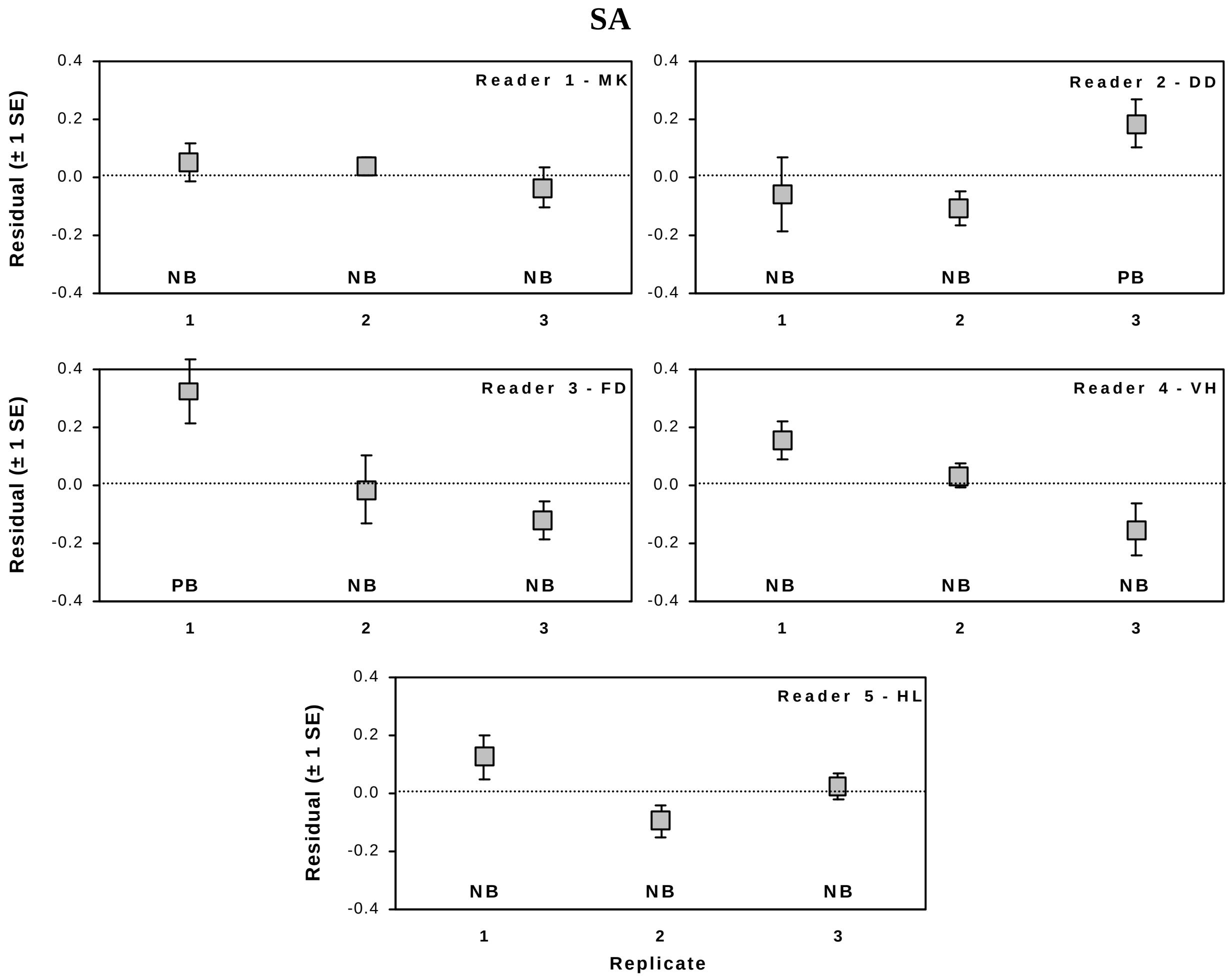

Figure 3b: Within-reader bias comparisons. Residual plots – **SA**. The average residuals (± 1 SE) are plotted for each of the three replicate readings by each reader. Each residual represents the average of the deviations of the age estimates from the modal age of each otolith. The results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated as no bias (NB), possible bias (PB) or certain bias (CB) for each replicate.

ANG

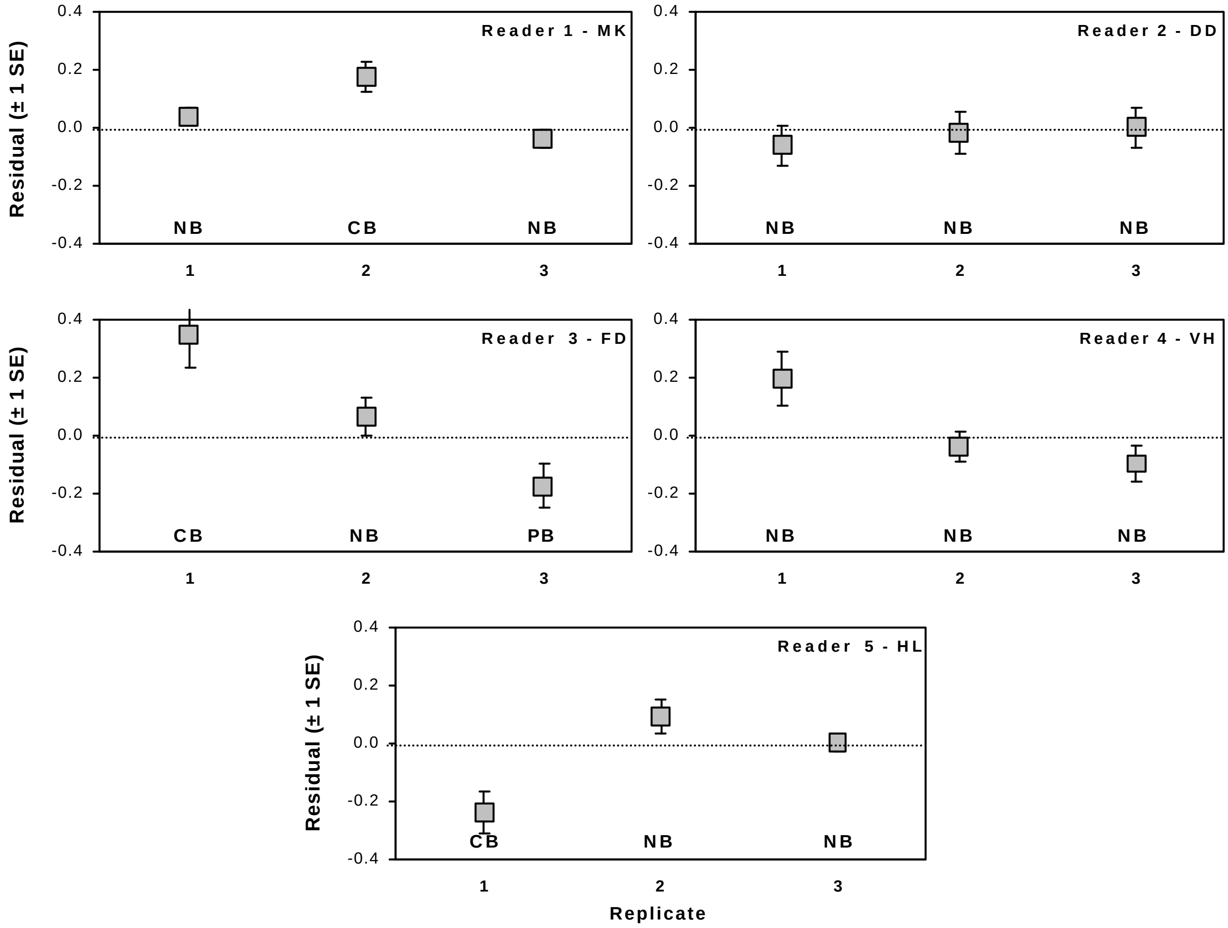

Figure 3c: Within-reader bias comparisons. Residual plots – **ANG**. The average residuals (± 1 SE) are plotted for each of the three replicate readings by each reader. Each residual represents the average of the deviations of the age estimates from the modal age of each otolith. The results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated as no bias (NB), possible bias (PB) or certain bias (CB) for each replicate.

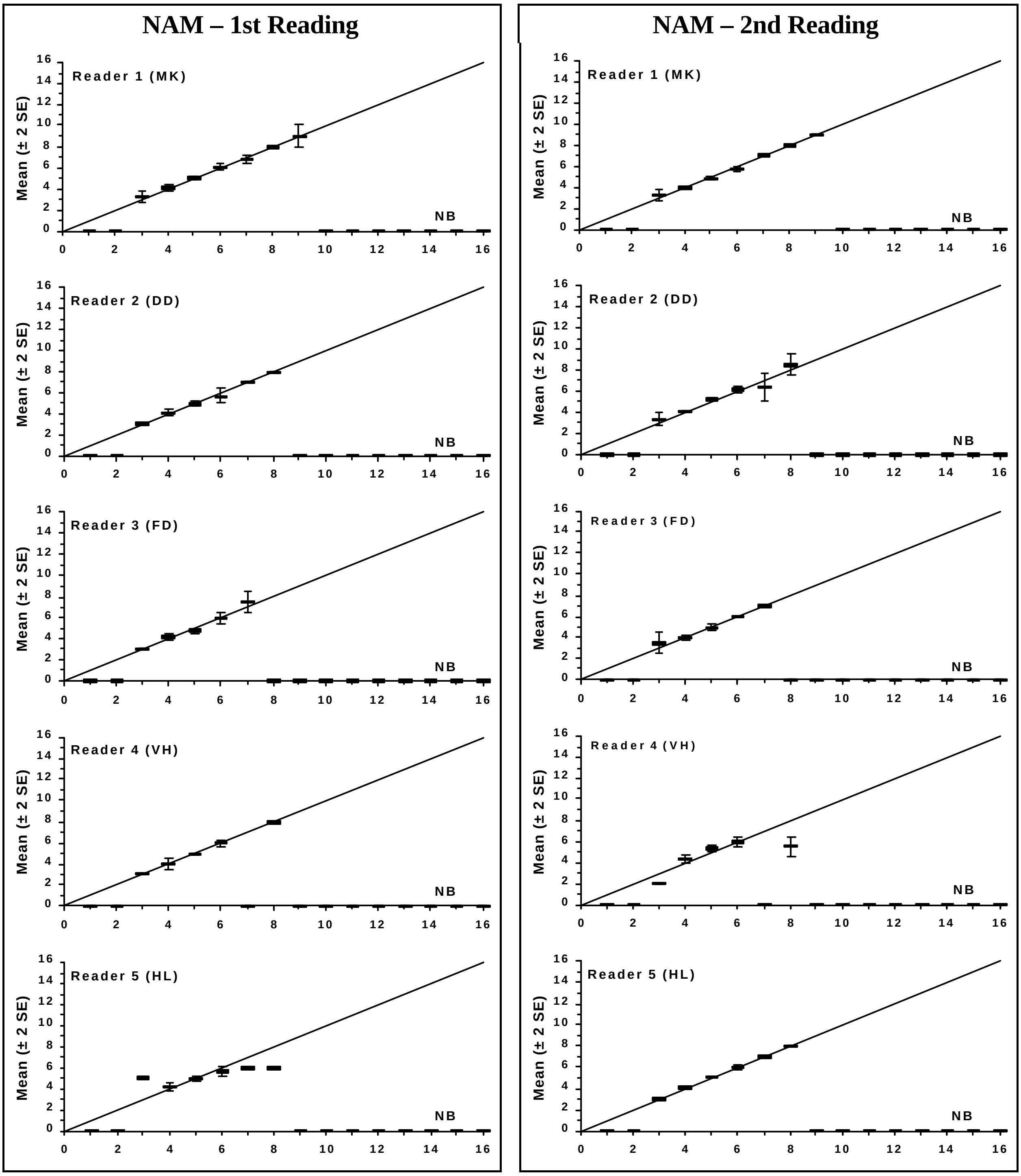

Figure 4a: Within-reader comparisons. Age-bias plots – **NAM**. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

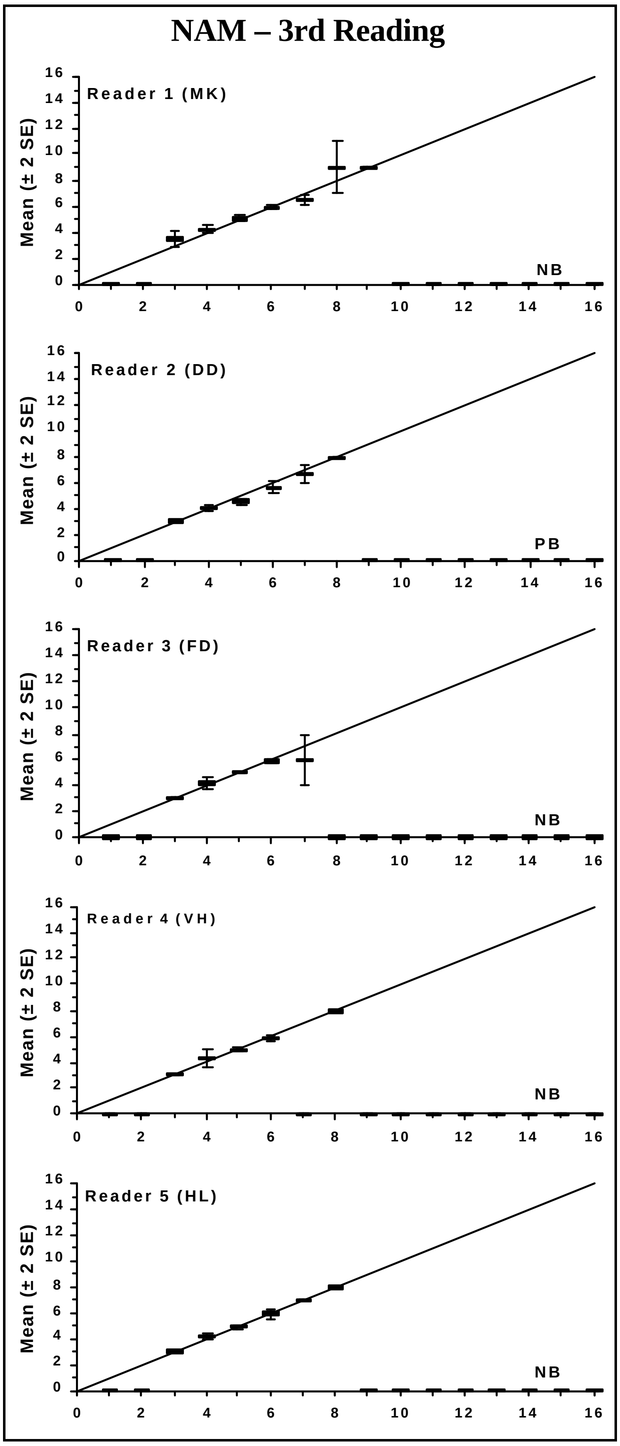

Figure 4a (continued): Within-reader comparisons. Age-bias plots – **NAM**.

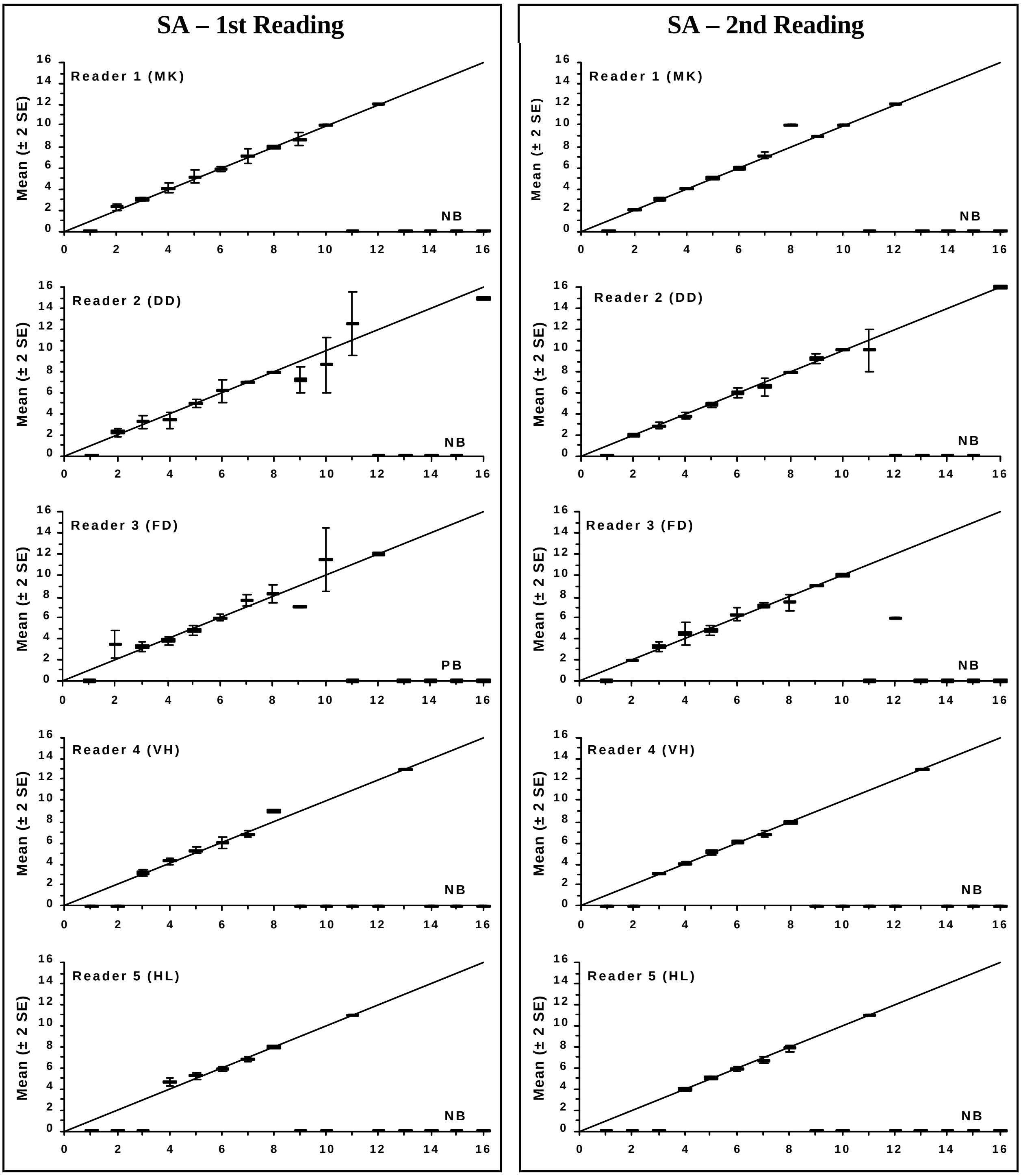

Figure 4b: Within-reader comparisons. Age-bias plots – **SA**. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

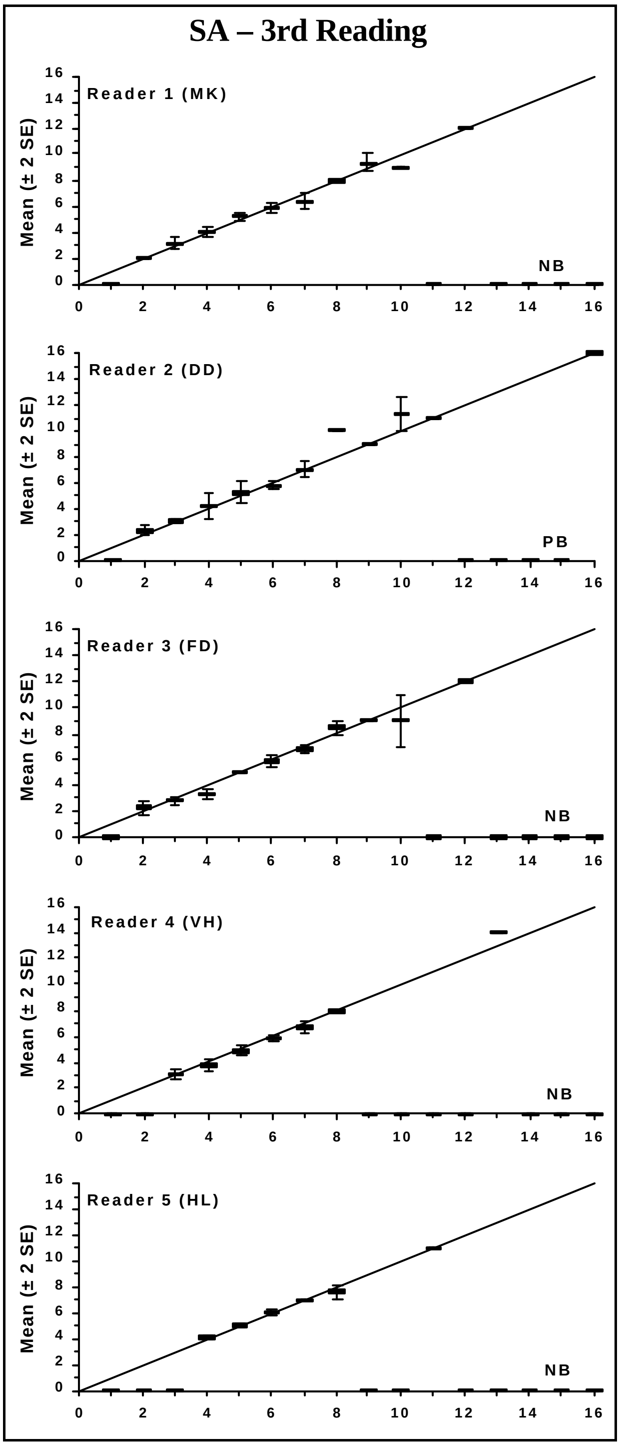

Figure 4b (continued): Within-reader comparisons. Age-bias plots – **SA**.

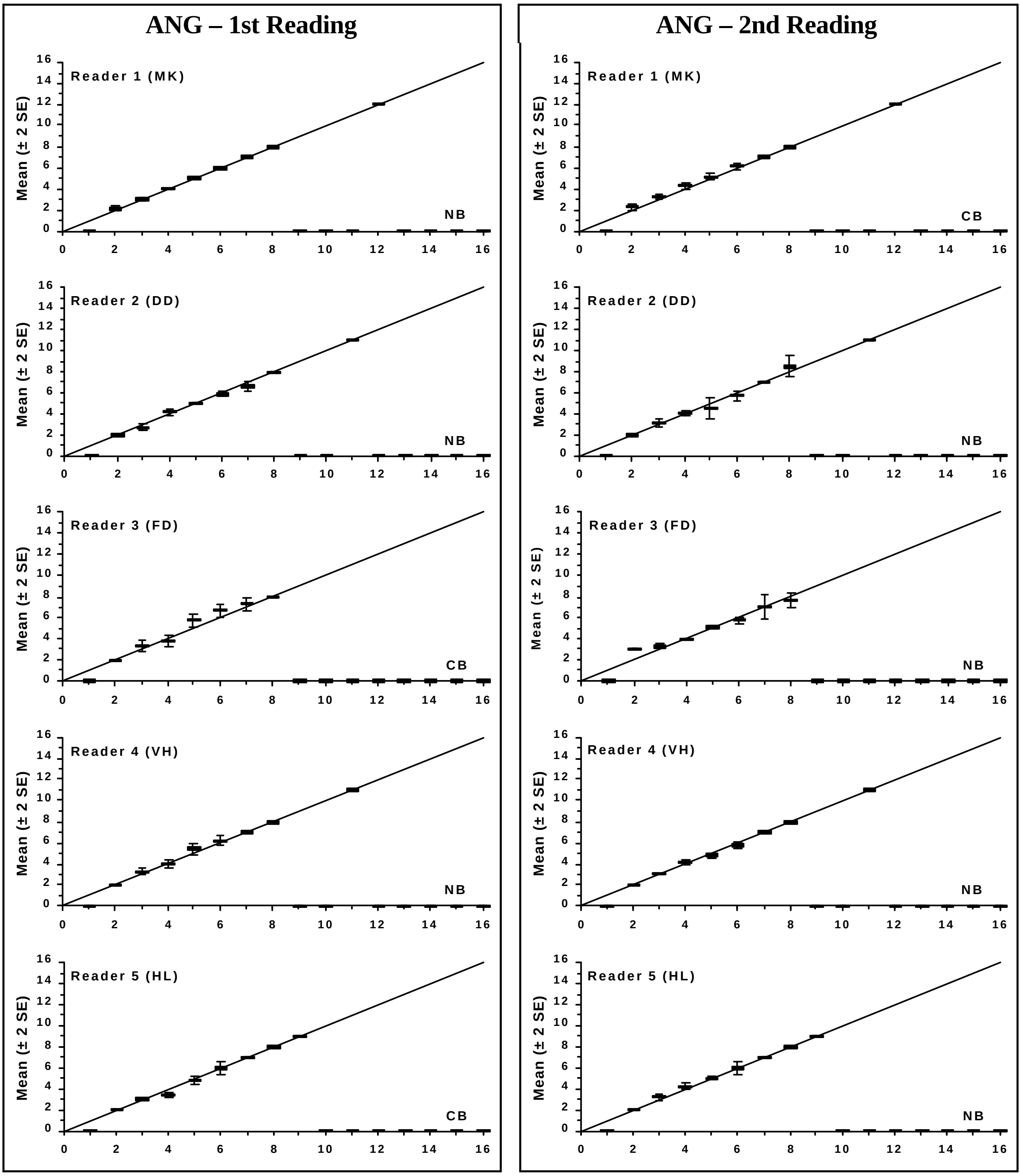

Figure 4c: Within-reader comparisons. Age-bias plots – **ANG**. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 2) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

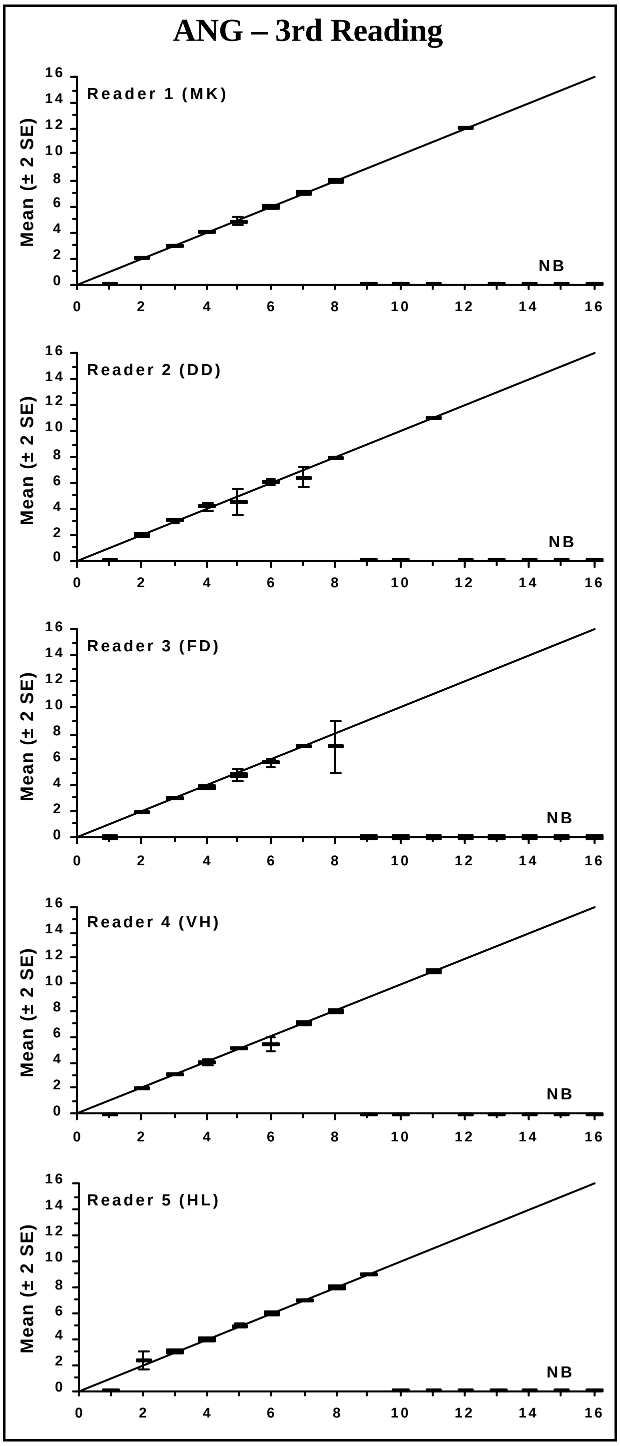

Figure 4c (continued): Within-reader comparisons. Age-bias plots – **ANG**.

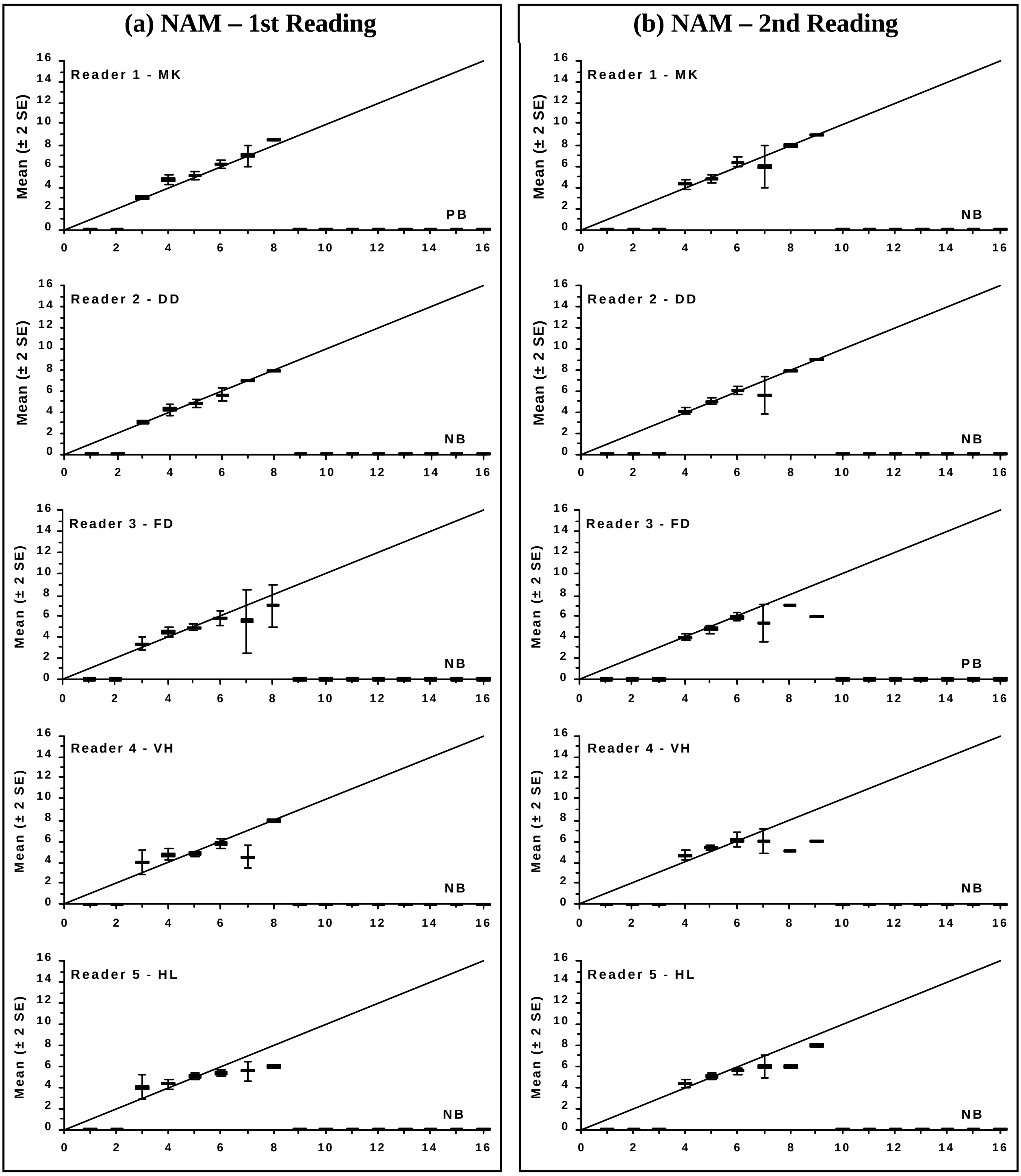

Figure 5: Among-reader comparisons (age-bias plots) - **NAM**. (a) First reading. (b) Second reading. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

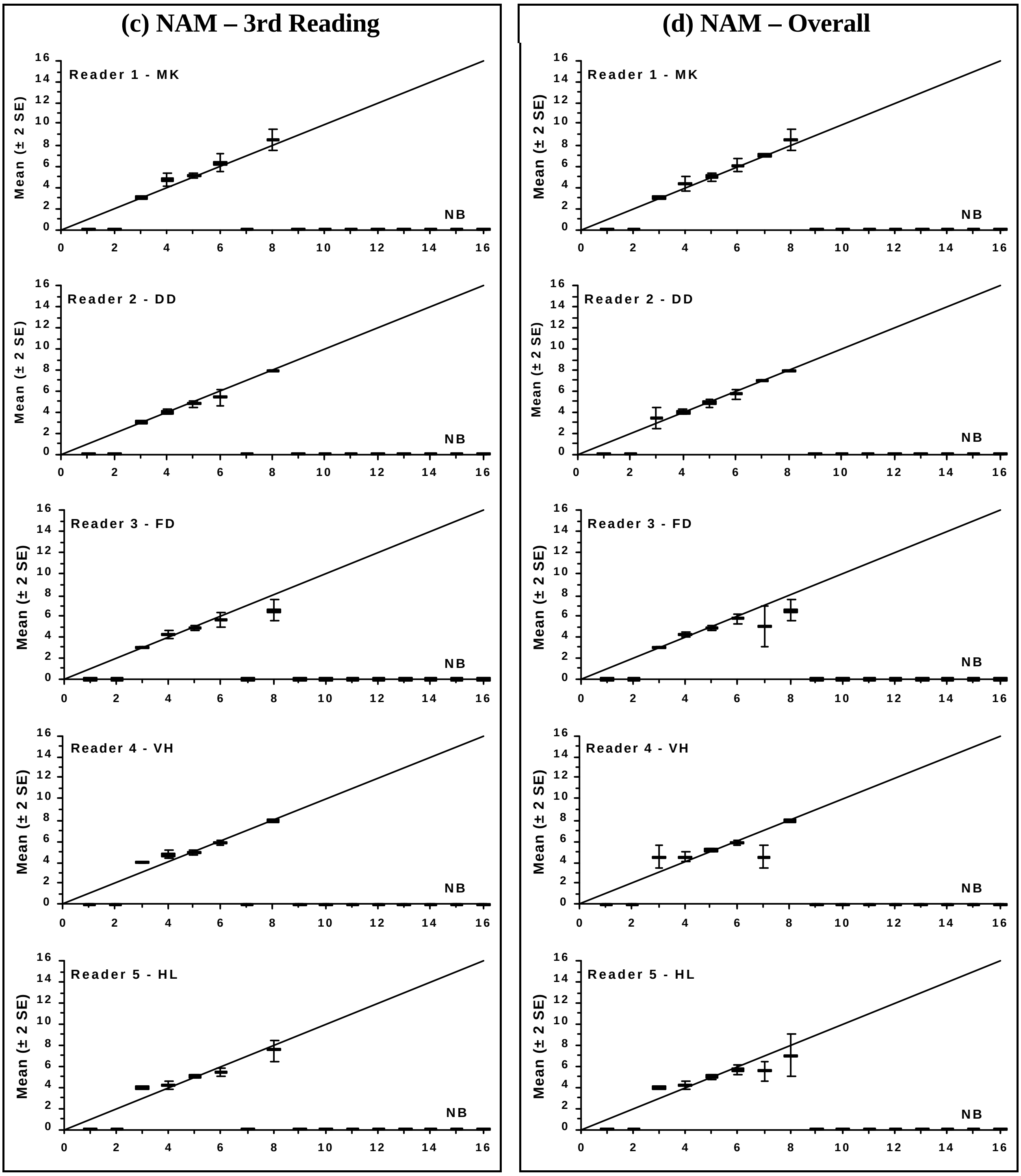

Figure 5 (continued): Among-reader comparisons (age-bias plots) - **NAM**. (c) Third reading. (d) Overall. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. In the case of the overall plots (d), the means of the modal ages computed for each reader are plotted against the modal ages computed from all fifteen readings by all five readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

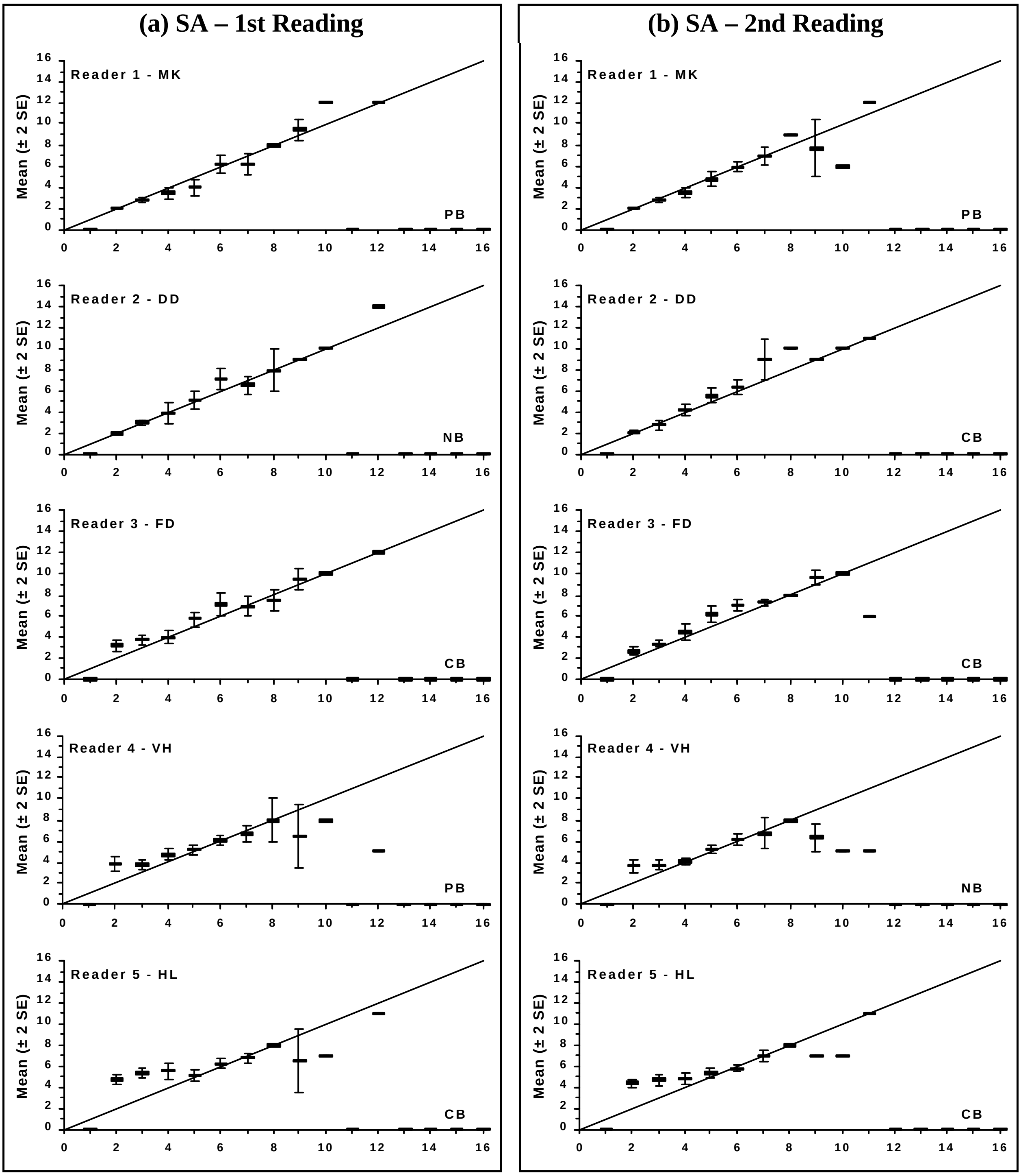

Figure 6: Among-reader comparisons (age-bias plots) - **SA**. (a) First reading. (b) Second reading. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

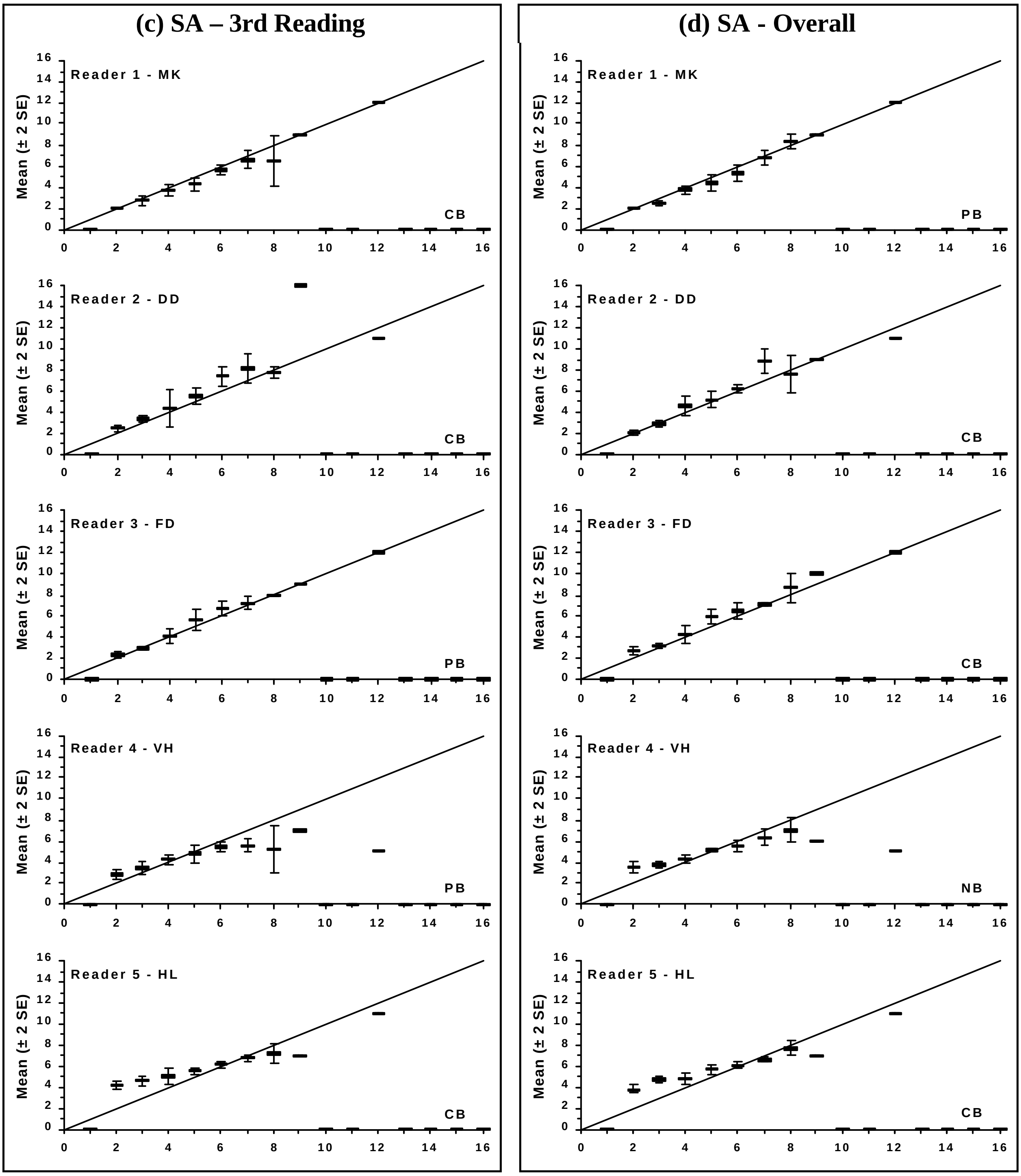

Figure 6 (continued): Among-reader comparisons (age-bias plots) - **SA**. (c) Third reading. (d) Overall. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. In the case of the overall plots (d), the means of the modal ages computed for each reader are plotted against the modal ages computed from all fifteen readings by all five readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

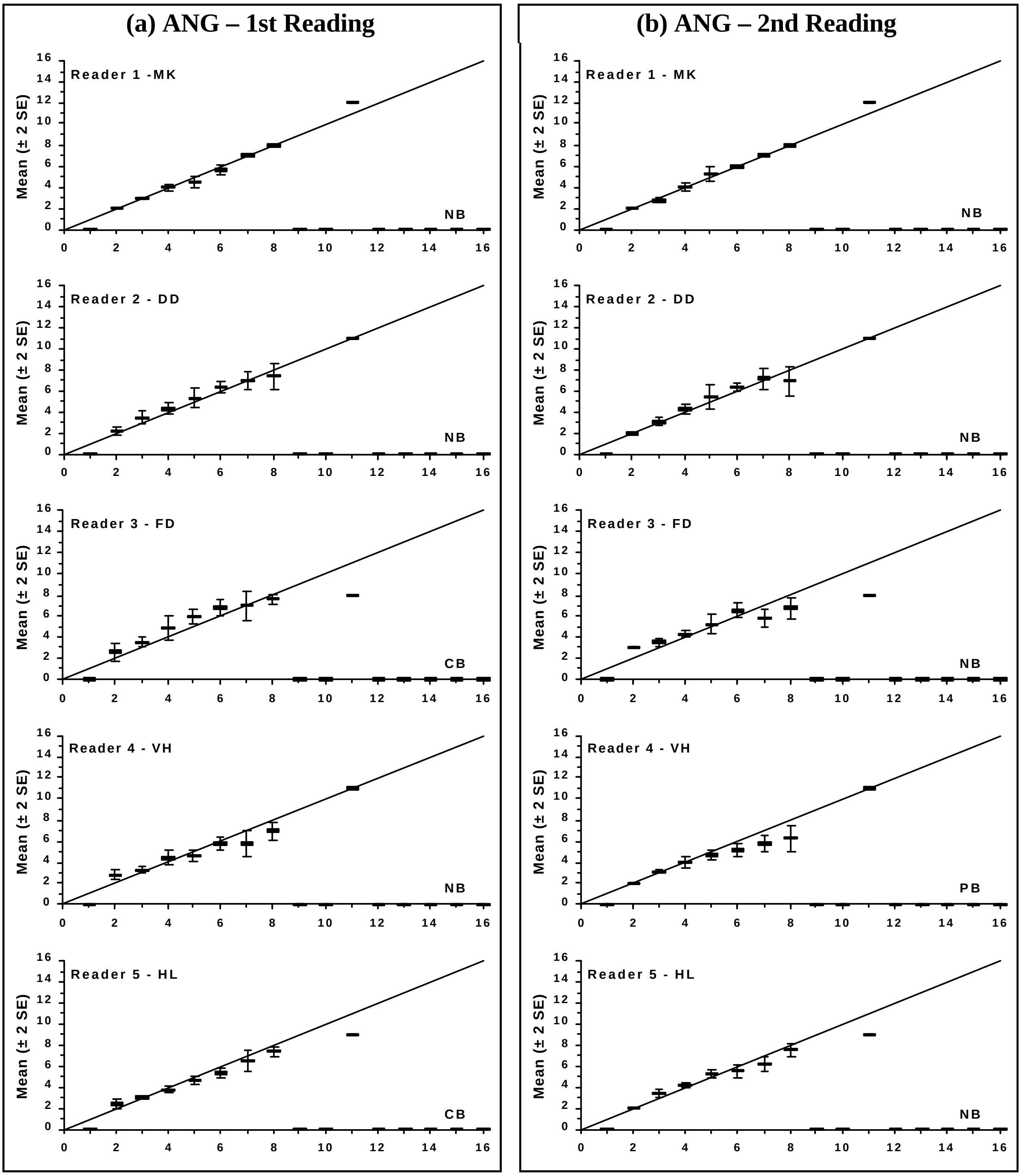

Figure 7: Among-reader comparisons (age-bias plots) - **ANG**. (a) First reading. (b) Second reading. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).

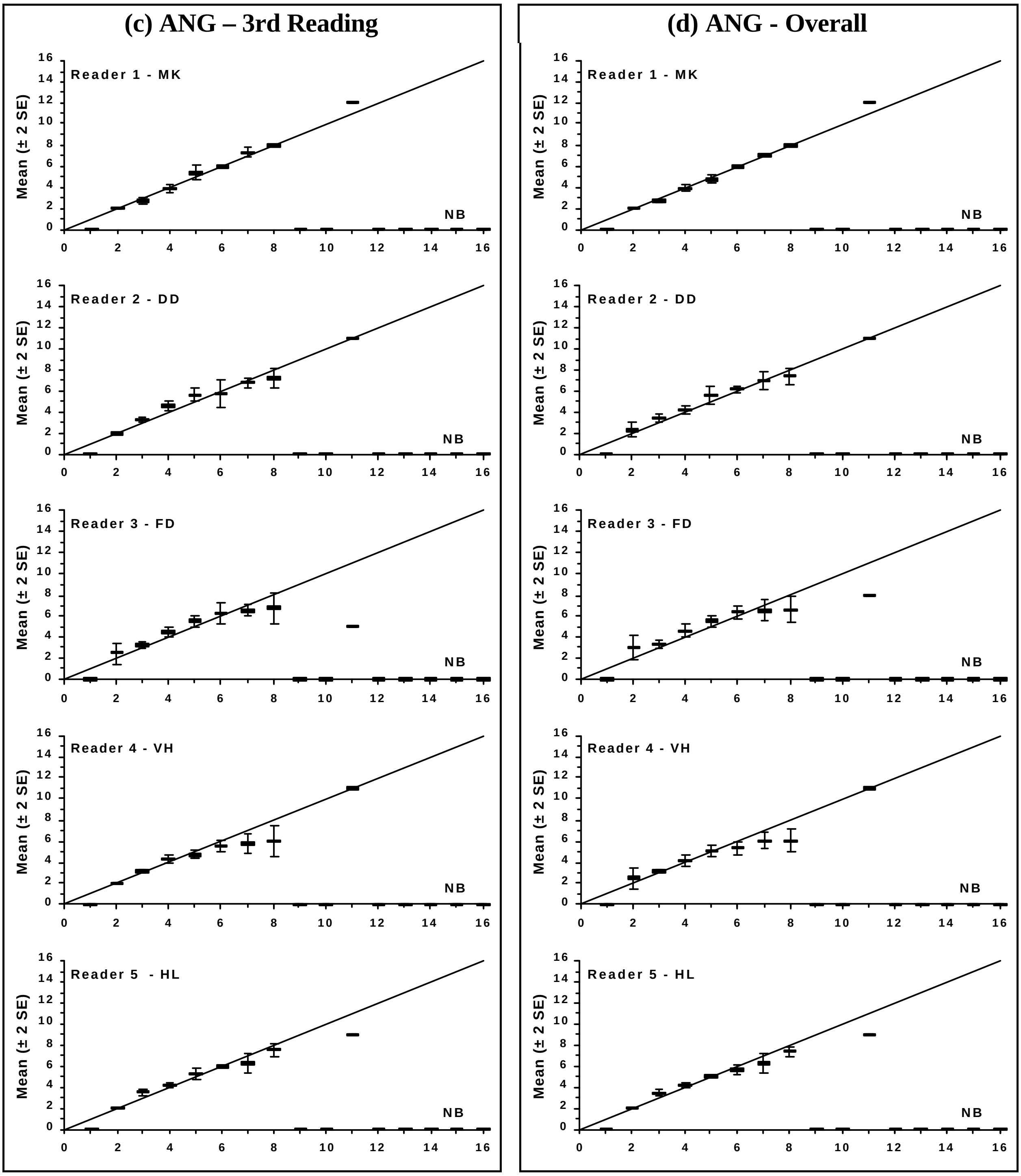

Figure 7 (continued): Among-reader comparisons (age-bias plots) - **ANG**. (c) Third reading. (d) Overall. The average (± 2 SE) of the age estimates recorded by each reader during each replicate reading are plotted against the corresponding modal ages computed from the five replicate readings by all readers. In the case of the overall plots (d), the means of the modal ages computed for each reader are plotted against the modal ages computed from all fifteen readings by all five readers. The 1:1 relationship illustrating no bias is shown with a solid line. Results of the Wilcoxon matched pairs rank tests (see Table 3) are indicated in each plot as no bias (NB), possible bias (PB) or certain bias (CB).