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Age readings of *Sebastes marinus* and *S. mentella* otoliths: bias and precision between readers

by

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Abstract

This study presents comparative otolith-based age readings of golden redfish (*Sebastes marinus*) from Iceland and demersal as well as pelagic deep-sea redfish (*Sebastes mentella*) from East Greenland and the Irminger Sea within an otolith exchange program between institutes in Germany, Iceland, Norway and Spain. A total of 571 otoliths were thin-sectioned and read independently in the participating labs. Age reading results were compared between readers in terms of bias and precision, using a set of statistical tests and graphical methods. Significant bias was observed between readers, mainly caused by deviations between age scores in the higher ages (> 20 years). Percent agreement was poor (< 30%) for a tolerance level of ± 0 years, particularly for the age range 21-40 years, which represents the major fraction of the fished stock. A tolerance level of ± 5 years, however, lead to up to 95% agreement for the age ranges up to 20 years. Precision and bias were generally better for *S. marinus* than they were for *S. mentella*, pointing to greater difficulties in the interpretation of growth stuctures for the latter species. The mean age of *S. mentella* with total lengths of 25-30 cm, which were observed to have recruited from the East Greenland shelf into the Irminger Sea during 1998/99, was determined to be 9-10 years. The observed problems in bias and precision of age readings should to be improved by continuing with similar *Sebastes* otolith exchange programs and setting up a further age reading workshop to harmonise the interpretation of growth structures. Radiometric ageing is currently being finalised and results will be available for the next NWWG meeting.

Keywords: redfish, golden redfish, deep-sea redfish, *Sebastes marinus*, *Sebastes mentella*; age reading, age determination; bias, precision, percent agreement

Introduction

Age determinations provide essential input data for the stock assessment of marine fish stocks. The age-based stock assessment of redfish (*Sebastes* spp.) in the North Atlantic, however, proved to be difficult due to the lack of a sufficient amount of reliable age readings. The reliability of a set of hard body structures of the fish was addressed several times in the past. Various studies (*e.g.* Chilton and Beamish 1982, Nedreaas 1990) and workshops (*e.g.* ICES 1991, ICES 1996) have shown that the otoliths are the preferred structure for age readings on North Atlantic *Sebastes* species due to an underestimation of older ages using scales and difficulties in the interpretation of other structures such as fin rays or vertebrae. Most laboratories are still reluctant to implement routine age readings on otoliths of *Sebastes* since there are concerns about the error observed in age reading results.

Age reading error has two major elements: bias and precision. The bias of age readings is caused by a consistent deviation of reading results between readers and is skewed from the mean to one side or the other, while precision of age readings measures the closeness of repeated independent age estimates (Wilson *et al.* 1987, ICES 1996). Precision reflects the degree of agreement among readers and is not to be confused with accuracy, which relates to the agreement with the true age of the fish.

Although there are routine testing systems and procedures for the assessment of bias and precision of age readings available (Kimura and Lyons 1991, ICES 1994, Campana *et al.* 1995, Hoenig *et al.* 1995, C.A.R.E. 2000), a broad-scale application of these methods in the laboratories carrying out redfish age readings is still missing. The 1995 ICES Workshop on Age Reading of *Sebastes* spp. (ICES 1996) showed a considerable bias between readers, which was shown to be improved after discussion of general interpretation of growth structures on the sectioned otoliths. Thus, the need for further exchange of material and knowledge on age reading methods was stressed.

As part of an EU-funded redfish project, an otolith exchange between redfish age reading experts of the participating institutions is being carried out to evaluate differences in age readings between readers and otolith preparation methods. The otolith exchange in 2000 was based on *Sebastes marinus* from the Icelandic shelf (Stransky *et al.* 2001). In 2001, the otolith readers comparison focused on pelagic *S. mentella*, sampled in the Irminger Sea during summer 1999. The *S. mentella* material exchanged in 2002 was collected on the East Greenland shelf and in the Irminger Sea during a period when recruitment of young *S. mentella* with total lengths of 25-30cm from East Greenland into the Irminger Sea was observed (October 1998 and June/July 1999, respectively; Stransky 2000). The age reading results of these otolith exchange schemes are presented in this study, with regard to bias and precision between readers. Extended results from the reading comparisons in 2000 (Stransky *et al.* 2001) and 2001 (Stransky *et al.* 2002) are presented since the scores of a fourth age reader (Spain) were included recently.

Materials and methods

The first set of otoliths used in this study were collected from *Sebastes marinus*, caught during the Icelandic groundfish survey in 1997, onboard M/V “Brettingur NS”, one of the participating vessels. The otoliths were taken from five hauls on the Icelandic shelf (ICES sub-area Va) on 14 March 1997. 212 otolith pairs were selected for age determination, covering fish of 10-54 cm in total length. From each pair, one otolith was prepared for age reading using the ‘break-and-burn’ technique (Christensen 1964, Chilton and Beamish 1982, MacLellan 1997), while the other otolith was thin-sectioned based on the technique described by Bedford (1983). The preparation by break-and-burn was carried out at the Marine Research Institute (MRI) in Reykjavik/Iceland. The rings in the otoliths were counted using different microscope magnification (maximum of 100x). The light was coming from above and a drip of glycerine was put on the otolith before counting the rings. The angle of the light was about 30-45 degrees. For the comparison of otolith preparation methods, the cross-sections were read by the same person at MRI who prepared and read the corresponding other otoliths by break-and-burn. The thin-sections were performed at the Institute for Sea Fisheries of the Federal Research Centre for Fisheries in Hamburg/Germany. A diamond-covered saw blade of 0.3 mm thickness and 100 mm diameter, rotating at 6000 rotations/min, was used to cut cross-sections of 0.5 mm thickness. The cross-sections were embedded onto glass plates with translucent polyester resin and read with a magnification of 150-200x using polarised transmitted light. At the Institute of Marine Research (IMR) in Bergen/Norway, the otolith cross-section plates were read trough transmitted light using a magnification of 50x.

The second set of otoliths used in this study were collected from *S. mentella*, caught within a commercial sampling program (EU Study 97/004 “Sampling of 8 German commercial fisheries”) in July 1999, onboard the German F/V *Fornax*. The otoliths were taken from twelve hauls in the Irminger Sea (ICES sub-area XII) at mean depths of 235-285 m. 213 otolith pairs were selected for age determination, covering fish of 22.5-41.5 cm in total length. From each pair, one otolith was prepared for age reading using the thin-sectioning technique described by Bedford (1983), while the other otolith was kept for later radiometric age validation (*e.g.* Campana *et al.* 1990). The otolith thin-sections were carried out at the Institute for Sea Fisheries of the Federal Research Centre for Fisheries in Hamburg/Germany. A diamond-covered saw blade of 0.3 mm thickness and 100 mm diameter, rotating at 6000 rotations/min, was used to cut cross-sections of 0.5 mm thickness. The cross-sections were embedded onto glass plates with translucent polyester resin and read with a magnification of 25-30x using polarised transmitted light. These otolith cross-section plates were read at the Marine Research Institute in Reykjavík/Iceland with transmitted light, magnified 40x. At the Institute of Marine Research in Bergen/Norway, the plates were read through transmitted light using a magnification of 20-35x.

The third set of otoliths encompassed 146 *S. mentella* otoliths, collected on the German FRV *Walther Herwig III* on the East Greenland shelf (October 1998) and in the Irminger Sea (June/July 1999), which were prepared and read by Reader 1 (for methods, see Stransky *et al.* 2001 and 2002). Subsequently, this material has been read by Readers 2, 3 and 4.

Age bias plots (ICES 1994, Campana *et al.* 1995, Eltink 1997) visualise the deviation of the age scores of two readers or methods from the 1:1 equivalence line and also allow the detection of non-linear bias patterns, *e.g.* the underestimation of ages by one reader in one part of the age range and overestimation in another part of the age range. The mean age assigned by one reader for all fish assigned a given age by the second reader is presented including the standard deviation around the mean. Various measures for precision were suggested for the comparisons of age readings. One of the more common indices is the percent agreement that compares the percentage of age determinations that are in agreement within a specified number of years.

Results

The age-bias plots for the *S. marinus* ageing comparison (Fig. 1) indicate relatively high non-linear bias, particularly from ages >20 years. In addition to the results presented in Stransky *et al.* 2001, reader 4 allocates higher ages in the range <13 years and lower ages in the range>13 years compared to all other readers. In most of the ageing scores of reader 4, ages >20 years are subject to high variation. The percent agreement plots (Fig. 2) illustrate the decreased correspondence of readings of reader 4 with the other readers, especially within the age range 0-10 years. For the medium age range (11-20 years), an agreement of 70-80% (depending on the reader pair) is reached with a tolerance of ±2 years.

Results from the expanded *S. mentella* readings (in addition to Stransky *et al.* 2002) indicate a similar relative underestimation of ages by reader 4 (Fig. 3) and show non-linear bias as well as high variation in readings of reader 4, especially for ages >20 years. The decreased precision of these reader pairs is clearly visible in the percent agreement plots for the age ranges 21-30, 31-40 and 41-50(50+) years (Fig. 4). The agreement between readers ranges between 35-66% for ±2 years and between 53-75% for ±3 years.

In 2002, the material was limited to *S. mentella* of 24-30cm (East Greenland shelf, Oct 1998; n=60) and 25-30cm total length (Irminger Sea, June/July 1999; n=86), respectively. These young redfish were observed to migrate from the shelf into the pelagic habitat during that period (Stransky 2000). Hence, the identification of the involved age-group(s) and the investigation of corresponding bias & precision was the task of this year's reading comparison, involving Partners 1, 2, 3 and 4. Fig. 5 shows the age-bias plots for this reading comparison. While the correspondence between readers 1 and 3 and readers 2 and 3 is relatively close, the other reader pairs, particularly the comparisons with reader 4, shows large bias, with the scores of reader 4 lying considerably lower than the readings of all other readers. Readers 2 and 3 generally scored pelagic *S. mentella* from the Irminger Sea 2-3 years older than *S. mentella* from the East Greenland shelf. Reader 4, however, grouped the majority of fish into a much smaller age range of 8-9 years. The agreement between readers (Fig. 6), applying ±2 years tolerance, varies from 62-98% for the East Greenland material and from 31-89% for the Irminger Sea material. Fig. 7 indicates some length dependence of ageing scores for readers 2 and 4. The distribution of ageing scores (Fig. 4.3.8) supports the division of East Greenland and Irminger Sea age ranges, most distinct for readers 2 and 3. For the calculation of the mean ages derived from these readings (Tab. 2), a narrow fish length range of 26.5-28.5 (East Greenland) and 27.5-29.5cm (Irminger Sea) was selected, representing the peaks of the observed length distributions (Stransky 2000) ±1cm. While the mean ages for the East Greenland samples range from 7.7-10.4 years, the mean ages for the Irminger Sea samples vary between 8.6-12.8 years. Reader 3 contributed the highest age scores, and Reader 4 the lowest scores to the mean ages. The extraordinary strong cohort present in the length distributions of juvenile redfish (*Sebastes* spp.) and *S. mentella* <20cm off East Greenland (Rätz and Sigurdsson 2002), however, are most likely fish from the 1991 year-class, so the ageing results of reader 4 are closest to this estimate (age 7 in 1998, age 8 in 1999). In contrast, 0-group indices from the Icelandic surveys (Magnusson and Johannesson 1997) did not match the observed strong cohorts.

Discussion

Further redfish otolith exchange

Considering the high bias in some of the traditional age readings as outlined above, especially for *S. mentella*, a series of digital pictures of otolith thin-sections will be taken during 2003 and every reader will be asked to set marks onto the proposed annuli. The comparison of these annotated pictures will help in explaining some of the observed bias and imprecision, *e.g.* due to different interpretation of the nucleus region, growth structures following the transition zone or in-/exclusion of marginal increments.

Radiometric ageing

The natural radioisotope radium-226 is incorporated into the otolith matrix where it decays radioactively to 210Pb (half-life 22.3 years). The ratio of the two radioisotopes is an index of the elapsed time since incorporation. By analysing the otolith cores, therefore, the age of the fish from the time of first incorporation (=birth) can be determined. Ra-226 and Pb-210 are determined by measuring the low-level radioactivity of 1g of otolith core matrix (3 first years) in alpha spectrometer counting cells for several weeks. Given the measured Pb-210/Ra-226 activity ratios and the corresponding radioactive decay constant, the otolith ages can be determined by a simple equation. These results will be compared to a series of independent optical age readings.

During 2002 and early 2003, the radiochemical separation Ra-226 in the otolith matrix was improved by implementing advanced methods for the determination of radium isotopes by Purkl & Eisenhauer (2002). A series of *S. marin*us and *S. mentella* samples is currently analysed for these radioisotopes and results will be available for the NWWG meeting in 2004.

Acknowledgements

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Table 1. Participating age readers.

Reader ID	**Name**	**Institute**
1	Christoph Stransky	Federal Research Centre for Fisheries, Institute for Sea Fisheries, Hamburg, Germany
2	Sif Guðmundsdóttir	Marine Research Institute, Reykjavík, Iceland
3	Svend Lemvig	Institute of Marine Research, Bergen, Norway
4	Fran Saborido-Rey	Institute of Marine Research, Vigo, Spain

Table 2. Mean ages of demersal *S. mentella* from the East Greenland shelf (26.5-28.5cm; n=26) and pelagic *S. mentella* from the Irminger Sea (27.5-29.5cm; n=55).

Mean ages (years)	All readers	Reader 1	Reader 2	Reader 3	Reader 4
East Greenland	9.30 (±1.14)	10.12 (±1.77)	8.96 (±1.34)	10.38 (±1.88)	7.73 (±1.04)
Irminger Sea	10.67 (±1.05)	10.56 (±1.52)	10.69 (±0.93)	12.78 (±2.42)	8.57 (±0.92)
Difference E-Greenland - Irminger Sea	1.37	0.45	1.72	2.40	0.84

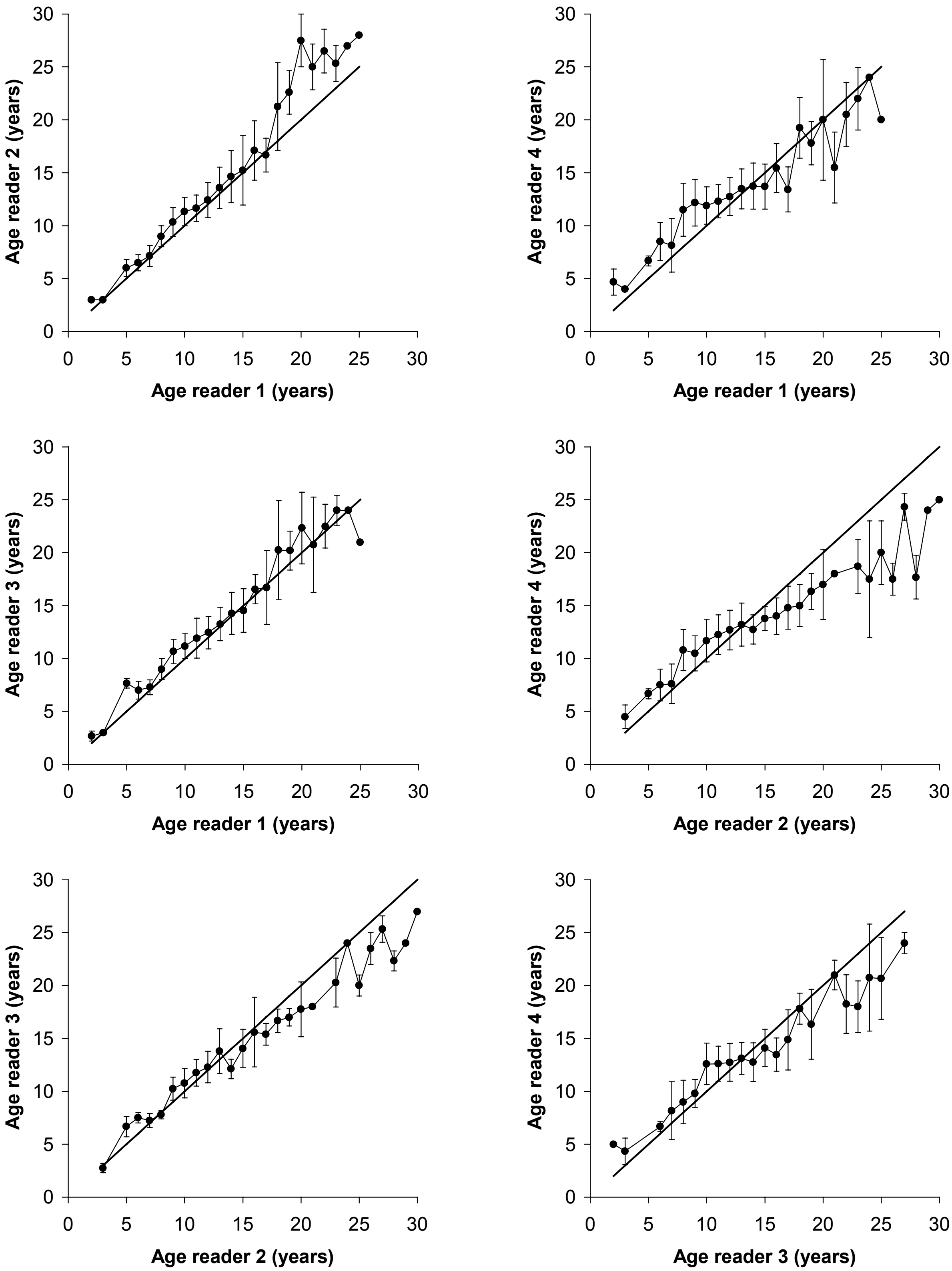

Fig. 1. Age bias plots for the reader comparisons based on the *S. marinus* otoliths from the Icelandic shelf (see Stransky *et al.* 2001, adding readings of Partner 2 carried out in 2002, Stransky *et al.* 2002). Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

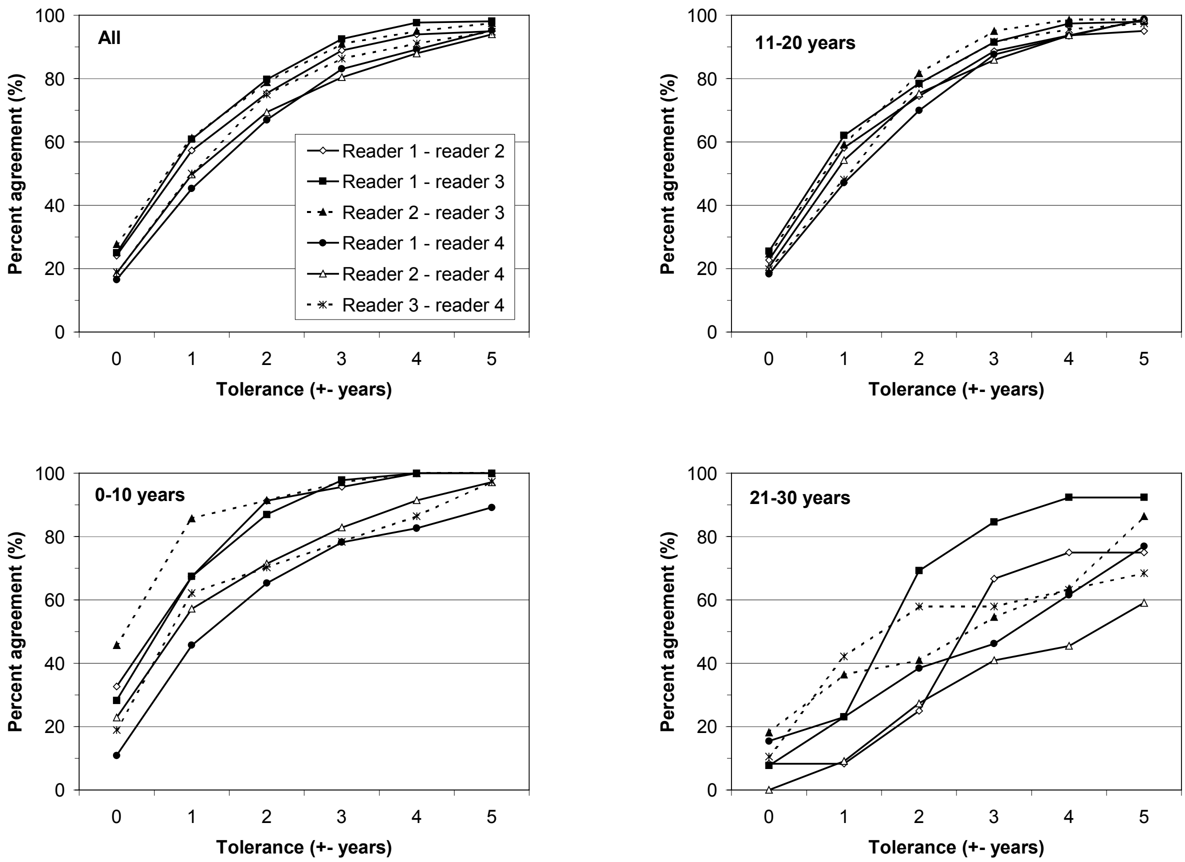

Fig. 2. Percent agreement plots for the reader comparisons based on the *S. marinus* otoliths from the Icelandic shelf (see Stransky *et al.* 2001, adding readings of Partner 2 carried out in 2002, Stransky *et al.* 2002) for a tolerance level (deviation of assigned ages between both readers) of ± 0 (total agreement) to ± 5 years. These were applied to all age groups and sub-sets of age ranges assigned by the first reader.

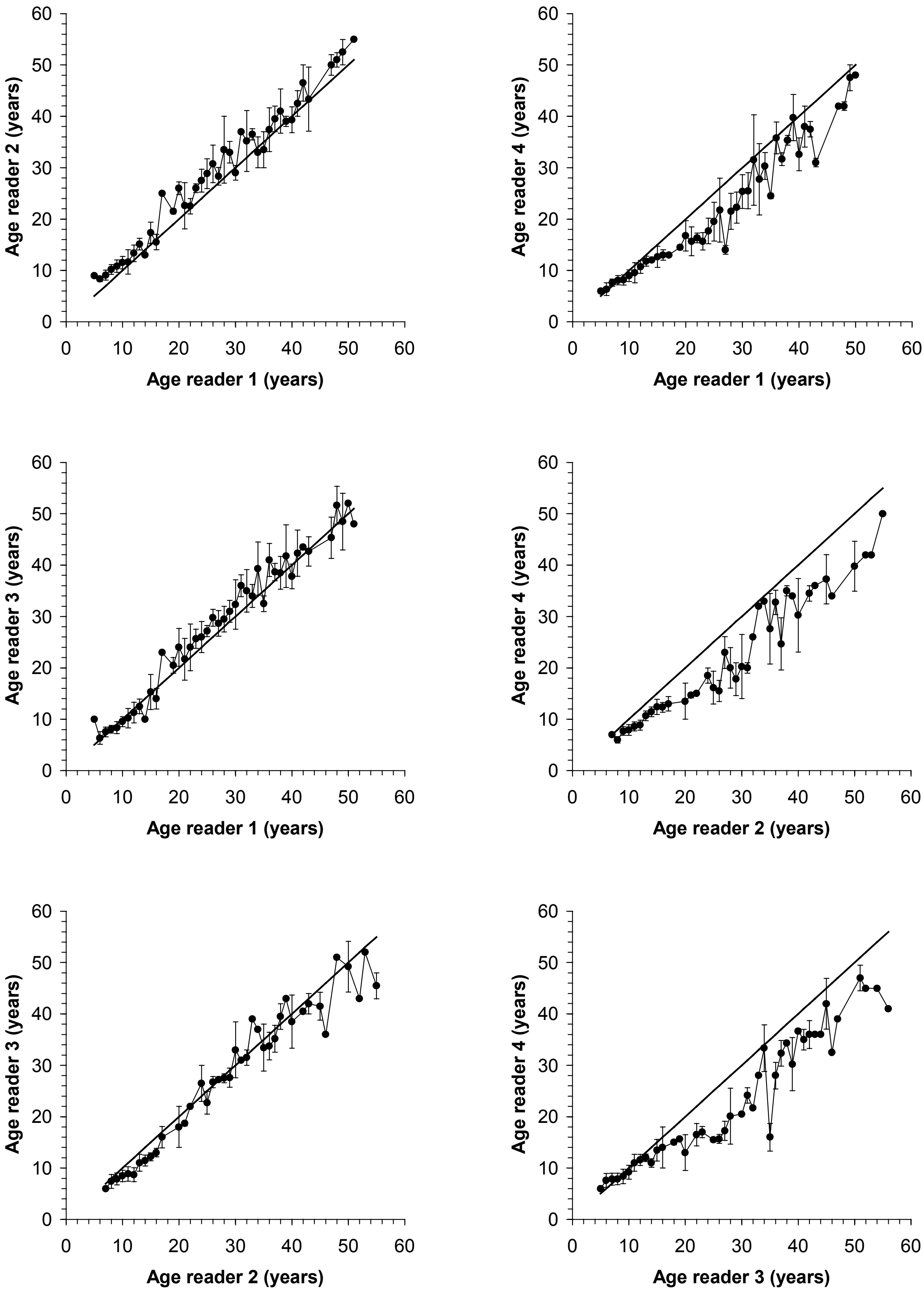

Fig. 3. Age bias plots for the reader comparisons based on the pelagic *S. mentella* otoliths from the Irminger Sea (see Stransky *et al.* 2001, adding readings of Partner 2 carried out in 2002, Stransky *et al.* 2002). Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

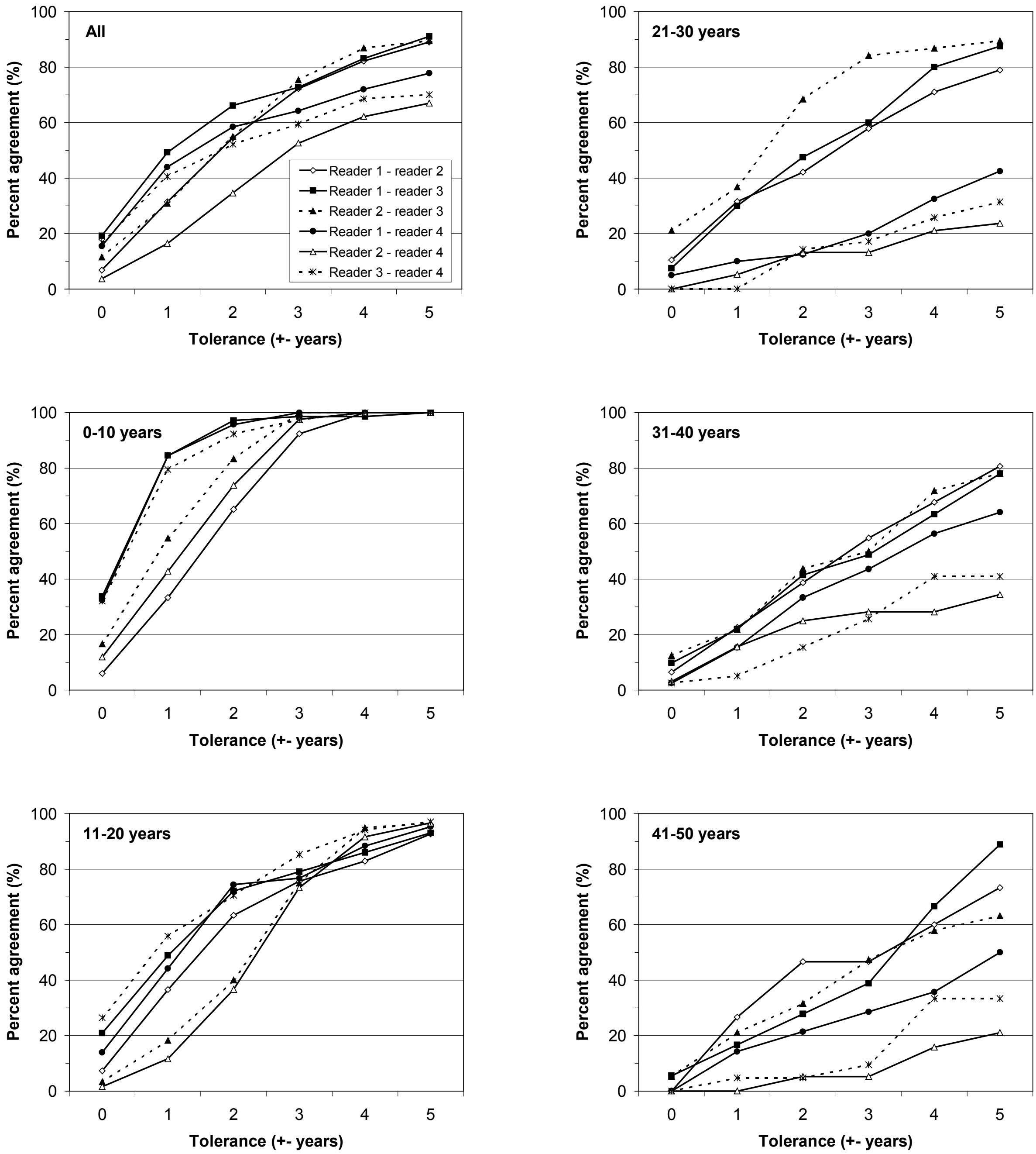

Fig. 4. Percent agreement plots for the reader comparisons based on the pelagic *S. mentella* otoliths from the Irminger Sea (see Stransky *et al.* 2001, adding readings of Partner 2 carried out in 2002, Stransky *et al.* 2002) for a tolerance level (deviation of assigned ages between both readers) of ± 0 (total agreement) to ± 5 years. These were applied to all age groups and sub-sets of age ranges assigned by the first reader.

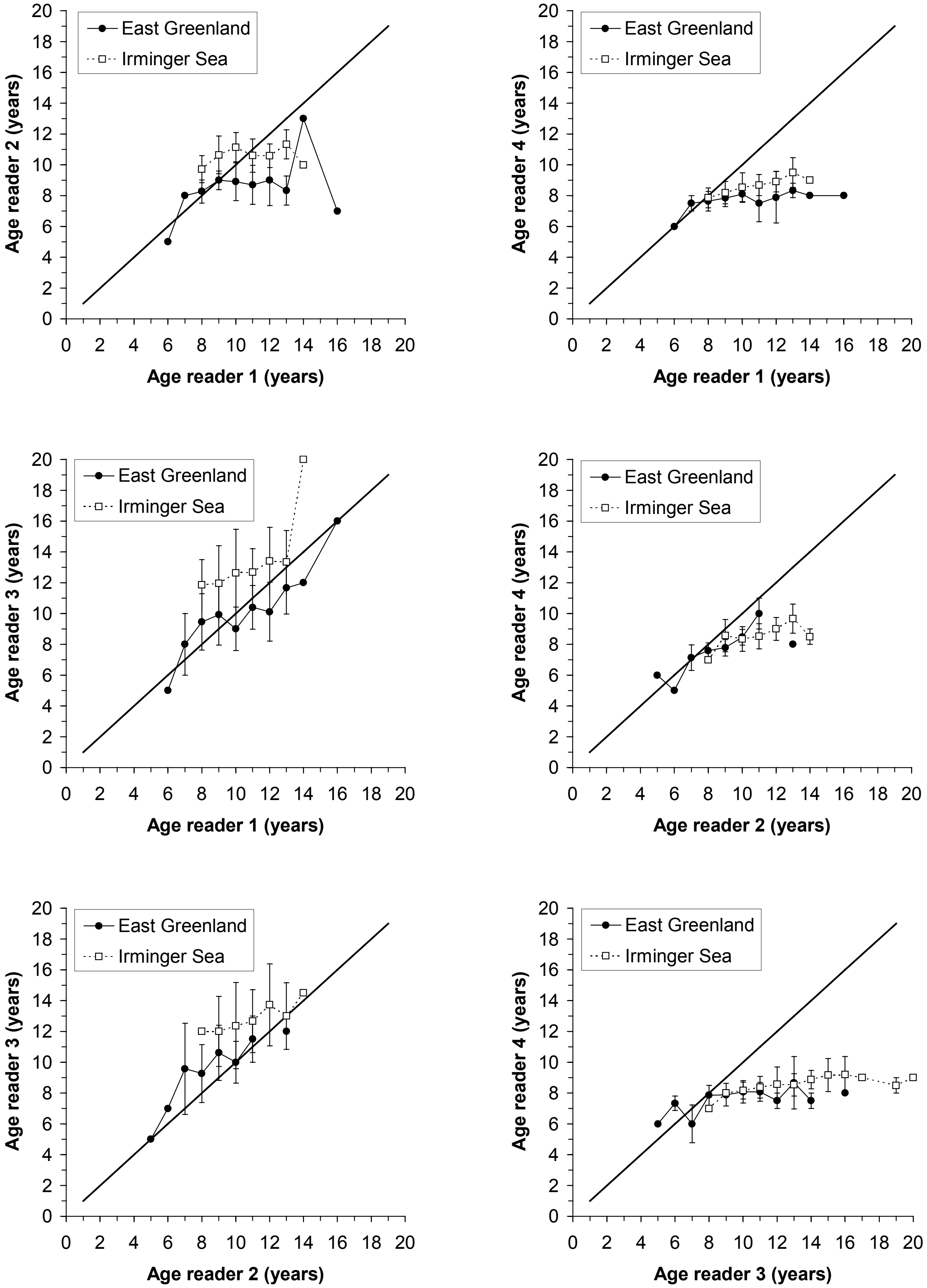

Fig. 5. Age bias plots for the reader comparisons based on otoliths of demersal *S. mentella* from the East Greenland shelf and pelagic *S. mentella* from the Irminger Sea. Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

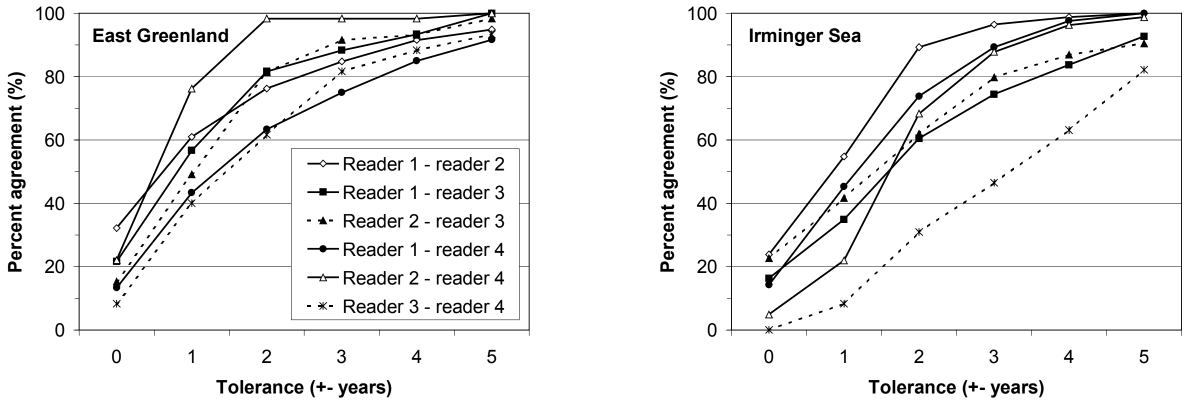

Fig. 6. Percent agreement plots for the reader comparisons based on otoliths of demersal *S. mentella* from the East Greenland shelf and pelagic *S. mentella* from the Irminger Sea for a tolerance level (deviation of assigned ages between both readers) of ± 0 (total agreement) to ± 5 years. These were applied to all age groups and subsets of age ranges assigned by the first reader.

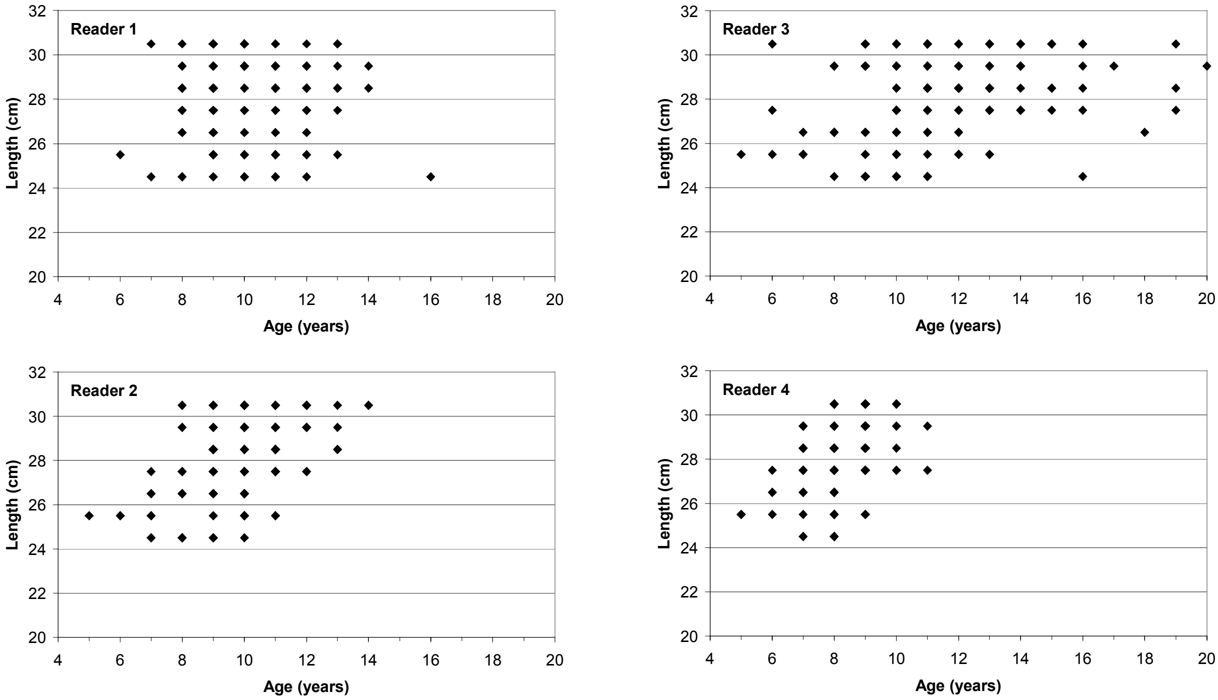

Fig. 7. Age reading scores for otoliths of demersal *S. mentella* from the East Greenland shelf and pelagic *S. mentella* from the Irminger Sea combined, illustrating the range and variation of readings and relationship with fish total length.

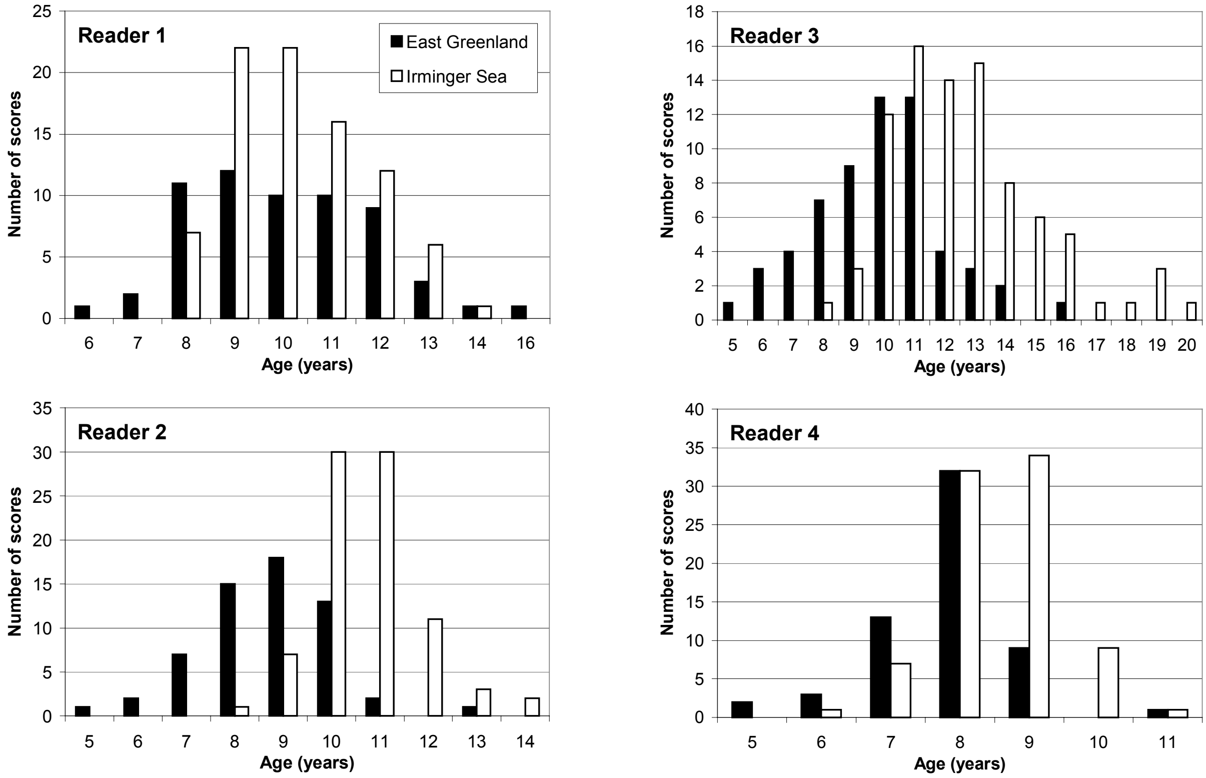

Fig. 8. Age distribution for otoliths of demersal *S. mentella* from the East Greenland shelf and pelagic *S. mentella* from the Irminger Sea.