A sand eel otolith age reading workshop was hosted by DIFRES in Charlottenlund, Denmark in September 2006. The objectives of the workshop were manifold; apart from the overall goal of securing consistency in age estimation of sand eel, updating and assembling age readers from all national laboratories handling sand eel from the North Sea to exchanged views on methods and experiences was among the objectives. This had not been done for more than a decade among the participating laboratories.

Prior to the workshop an otolith exchange was undertaken and the results were discussed during the workshop. The otolith exchange set consisted of 920 otoliths selected from commercial catches taken from the major Danish fishing areas in the North Sea during 2004. Sampling dates were evenly distributed over the months April through June. The overall agreement was 83.5% with a precision of 19.7% CV and in 53% of the otoliths the agreement was larger then 90%. The relative bias were not skewed for any ages (figure 1), although there were a slight, but not significant, tendency to overestimate the younger ages and overestimate the older compared to modal age. The two most experienced readers in the exchange had a consequent pattern of disagreement, where one reader (R1) were interpreting the age 1 year younger than the other in 42% of the individuals compared to the other reader (R2).

These discrepancies in interpretation of age structures in the otolith were further explored in an image analysis calibration during the workshop. The calibration exercise was a combination of a traditional age calibration exercise and an image analysis system approach.

The calibration otolith set consisted of 102 otoliths selected from Danish commercial samples from the Dogger Bank and Jyske Rev areas in the North Sea in April, May and June 2004. The analysis of the results was performed using an Excel ad-hoc Workbook “AGE COMPARATIONS.XLS” from A.T.G.W. Eltink from RIVO following the recommendations of EFAN (Eltink et al., 2000). Modal age was reached for all otoliths in the calibration set. The image analysis age calibration was performed using both ‘live’ otoliths under the stereomicroscope and digitized images of the corresponding otoliths. The readers had the otolith exposed under the stereomicroscope while pointing at the age structures on the picture using the image analysis system tool and could consult the ‘live’ otolith if the pictures did not reveal all the desired otolith structures clearly. The image analysis system tool makes use of XY-coordinates corresponding to the points, the reader marks as age structures on the digitised image of the otoliths. Prior to the exercise the readers agreed on one axis from the centre and towards the edge along the rostrum along which all points should be placed.

The overall agreement in the calibration exercise was somewhat lower than in the exchange (72.5% with a CV of 21.2%). This however should be taken with some caution as the participants in the exercise counted two new readers who did not participate in the exchange and then the two experienced readers, who did participate in the exchange. Thus an additional comparison of the results between the exchange and the calibration exercise only including the two experienced readers was performed and that showed an increase in percent agreement from 52% to 67% just between the two readers. The pattern in disagreement was persistent as R1 was identifying fewer age structures in the otoliths compared to R2.

The omission of age structures by the individual readers did have a pattern, thus it was possible to direct the discussion of which age structures to count towards the conception of ‘false rings’. Some otoliths showed to be very difficult to reach a common interpretation of the age and the points
counted as age structures were scattered along the otolith, however some trends were obvious and figures 2A and B shows the most typical patterns of the selective interpretation of age structures. The ‘false’ ring most frequently appeared when a second opaque zone had been formed during the first summer by some individuals, thus the definition of the first annual structure showed to be of high importance for reaching agreement on the age of the individual fish. However, also between the first and second year of growth the appearance of a ‘split-ring’ structure were the cause of discrepancy between readers. The most frequent argument for omitting a ring as ‘false’ was the width of the structure, if it appeared less wide than the remaining transparent structures it was considered false by some readers.

The image analysis exercise clarified that the lack of agreement can be referred to two reasons, the first being the position of the first ring where a secondary period of growth has been taken place during summer. This is often seen in the younger individuals as the otolith is thinner and thus the structures more clear. The second reason to disagreement arose where some readers choose to leave out specific rings identified by other readers as true annual rings where the rings successive to the 2nd ring were split rings.
Validation of annual structures by otolith microstructure appearance showed to be very useful for reaching agreement in the majority of the otoliths, where the readers did disagree. Inclusion of this method in the routine work with sand eel otolith when a reader is in doubt of the character of the age structures would be desirable. It will be a part of the standard set up in one of the ageing labs that participated in the workshop.

The two most experienced readers (R1 and R2) reached a high agreement through the course of the workshop and the training of the new readers would be done following the agreements from the workshop thus facilitating a continued high agreement between ageing labs despite the change of personnel.

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, some of the major problems in ageing otoliths of sand eel. The group reached agreement on an outline of ageing protocol/guidelines as described in section 5 of the present report and the aim is to produce a DVD training package, including extensive photo-documentation of otoliths with agreed and validated age structures by area and sampling month. This would be part of a reference collection for each area where actual otoliths and digitized images are available for training and future workshops.
Figure 1. The distribution of the age reading errors in percentage by modal age as observed from the whole group of age readers in an age reading comparison to modal age. The achieved precision in age reading by modal age group is relatively high as the spread of the age readings errors is narrow. There appears to be no relative bias, as the age reading errors are normally distributed.

Figure 2 (A) Individual nr. 27. From June, location 39F7; ages set as either 1 or 2- (B) Individual nr. 66: From April, location 39F1; ages set as 2, 3 or 4.