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REPORT OF THE ICES WORKSHOP

ON

SANDEEL OTOLITH ANALYSIS

Arendal, Norway 28 August - 1 September 1995

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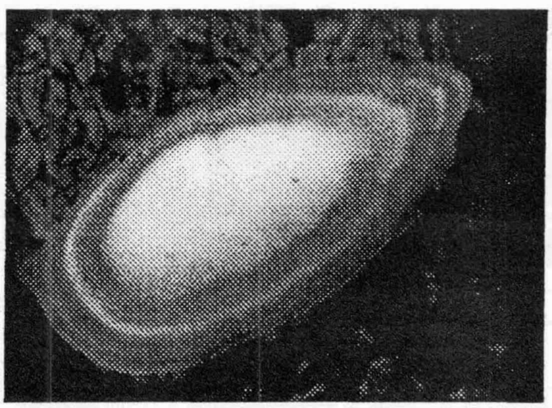

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* General Secretary
ICES
Palægade 2-4
DK-1261 Copenhagen K
DENMARK

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1. Introduction

1.1 Terms of Reference

At the 1994 Statutory meeting the following recommendation was made:

A Workshop on Sandeel Otolith Analysis will be held in Flødevigen, Norway from 28 August - 1 September 1995 under the chairmanship of Dr. E. Moksness (Norway) to:

a) describe a protocol for handling sandeel otoliths including preparation of 0-group otoliths for counting of day-rings;

b) conduct comparative age determinations and evaluate the results using the methods described by the Workshop on Sampling Strategies for Age and Maturity;

c) describe differences in otolith types with special reference to secondary rings and geographical differences in growth patterns;

d) establish a protocol for the age determination of otoliths using diagrams and photographs to illustrate age reading criteria.

1.2 Participation

The meeting was attended by the following:

Name	Country
Palle Brogaard	Denmark
Petter Fossum	Norway
Susanne Hansen	Denmark
Inger Henriksen	Norway
Henrik Jensen	Denmark
John Lahn-Johannessen	Norway
Harald Larsen	Norway
Erlend Moksness (Chairman)	Norway
Henrik Mosegaard	Denmark
Lisbet Solbakken	Norway
Peter Wright	UK (Scotland)

2. General

2.1 Review of sandeel biology

Sandeels belong to the family *Ammodytidae* and are small, elongate fish with a cylindrical or slightly laterally compressed body covered by small cycloid scales. The head is pointed with an anteriorly projecting lower jaw and, except in Hyperoplus, a protrusible pre-maxilla. The dorsal and anal fins are elongate, the caudal fin forked and pelvic fins are absent. In the North Sea and adjacent waters 5 different species occur regularly: The lesser sandeels (*Ammodytes tobianus* and *A. marinus*), the Smooth sandeel (*Gymnammodytes semisquamatus*), the Greater sandeel (*Hyperoplus lanceolatus*) and Corbin's sandeel (*H. immaculatus*). Reay (1970) provides information on sandeel taxonomy. *A. tobianus* and *A. marinus* are difficult to distinguish and meristic and morphological features provide the most reliable method for separating these species. Though slightly overlapping the total vertebral number, the dorsal fin ray number and the anal fin number are significantly higher in *A. marinus* than in *A. tobianus*.

Following larval metamorphosis sandeels are mainly found in association with sandy substrates into which they burrow. In addition to sediment type, depth and flushing rates appear to be important factors influencing sandeel distribution. *A. tobianus* tends to be found in depths of < 20 m, whilst *A. marinus* generally occurs in deeper waters on sand banks between 20 and 150 m depth. Other species of sandeel occur throughout the depth range of *A. tobianus* and *A. marinus*. Lesser sandeels can form very large schools and generally feed in the water column on plankton, such as copepods. Sandeels of the genus *Hyperoplus* are piscivorous and often feed on lesser sandeels.

Recent estimates of species composition of sandeel fisheries in the North Sea indicate that *A. marinus* account for approximately 90 per cent of the total sandeel landings and may be assumed to be sampled accordingly. Consequently the present workshop has concentrated on the biology of this species. The geographic range of *A. marinus* extends from the Barents Sea to Iceland south to the English Channel. For the purposes of assessment 3 stock divisions are recognised in the North Sea; the Northern, Southern and Shetland (Anon., 1995a). These divisions were initially based on regional differences in size at age of *A. marinus*. However, these growth differences are no longer apparent. Further, recent studies of 0-group distribution and protein polymorphisms suggest that the assessment divisions do not reflect reproductively isolated stocks (Wright, in press; Verspoor et al., 1995).

A. marinus generally spawn between December and January (Gauld & Hutcheon, 1990) although spawning may be as early as November in the Barents Sea (Andriashev, 1964) and as late as April in the English Channel (Corbin and Vati, 1949). Sandeels lay demersal eggs, which are covered in a glutinous secretion causing them to adhere to surrounding sand grains. Embryonic development is generally protracted and dependent on oxygen concentration and temperature. Larvae hatch from January - April, and the larvae are planktonic. Estimates of the duration of the larval phase in the north west North Sea range from 2 - 4 months, based on daily increments (Wright, in press). Larval metamorphose within the size range 35-55 mm TL. Many larvae hatch in areas where they are liable to be subject to rapid advection (see Hart, 1974; Wright, in press). However, there is no evidence that *A. marinus* larvae are transported to suitable nursery areas and the range in larval distribution is generally far greater than that of adults (Hart, 1974). Macer (1965) proposed that late larvae or juveniles must actively seek suitable grounds based on the sudden appearance of small juveniles at sand banks. During the period immediately following larval metamorphosis 0-group sandeel distribution can be very extensive and settlement appears to extend from late May through to August. 0-group may occur in commercial industrial catches from late June to early July onwards. Evidence from tagging studies suggest that once settled sandeels show a high degree of site attachment. Post-settled sandeels show a diel pattern of emergence from the sand in the spring and summer during which time they actively feed and grow (Wright & Bailey, 1993). The growth rate in length of the successive age groups as well as the fat content is highest in the second quarter of the year. Sandeel availability to fishing gears declines throughout the late summer and autumn possibly because of a reduction in emergence time (Wright & Bailey, 1993; Reeves, 1994). Except for spawning, sandeels appear to spend most of the period between October and March in the sand. Commercial sample age compositions suggest that the 1-group emerge in March or early April, earlier than the older age-classes.

Sexual maturity appears related to size (Gauld and Hutcheon, 1990), with most sandeels attaining maturity two years, although in some years a high proportion of 1 year old fish may mature. In general, the maximum age for *A. marinus* encountered from otolith readings is nine years.

In 1994 the Working Group on the Assessment of Norway Pout and Sandeel recognised that doubts over the reliability of age readings of sandeel otoliths was a recurrent problem. The main difficulties occurred in the interpretation of possible secondary rings and the termination of the translucent winter rings. In order to improve matters both within and between laboratories involved, the WG strongly felt the need for a workshop on age reading of sandeel to be established.

2.2 Objective of Workshop

The objective of this Sandeel Otolith Analysis Workshop are:

A) To produce guidelines which when applied minimise between-reader bias and variability in the precision of age determinations (i.e. their reproductibility).

B) To advise on what age groups can valid age readings be achieved.

C) To produce a protocol for handling sandeel otoliths.

D) To produce a protocol for the age determination of sandeel otoliths.

E) To produce procedures for the preparation and interpretation of otoliths at the daily level.

3. Material and Methods

In the report of the Workshop on Sampling Strategies for Age and Maturity (Anon, 1994), guidelines have been given to conduct age reading workshops. According to the guidelines, the following was included in the agenda: review of the biology, review of sample processing methods used, discussion on age determination criteria, age reading exercise and analysis of these.

Review of the sample processing methods used.

Preceding the workshop a questionnaire concerning sampling and sample processing was distributed among the participating laboratories from Denmark, Norway and Scotland. Questions were arranged in sixteen categories, covering the following subjects: sampling strategy, measurements taken, identification of species, dissection, types of otoliths taken, fate of abnormal otoliths, other analyses besides ageing, preparation of otoliths, methods for the observation of age structures, data relevant for age determination, criteria used for identification of annual increments, additional information used to aid age determination, features to be discussed at the WS, definition of increments, data retrieval, and comments.

<u>Sandeel sampling</u>
Samples of sandeel sagitta otoliths are taken from industrial samples from landings and during special sandeel surveys. Danish samples for ageing covered about 100 catches in 1994 from about 650.000 tonnes of commercial landings. About 60 samples with between 20 and 150 individuals each, were processed to give detailed information about length at age distributions. As only one sample per landing is taken there is no information about catch variability. Norwegian samples are selected to represent different areas and times of the year amounting to about 12 to 20 per year. Scottish samples cover every fishing ground, every day of the season with two samples. Depending on fishing activity and landings this amounts to between 12 and 250 samples per year. An average of 60 fish per sample is processed for length at age determination.

<u>Sandeel measurements</u>
All countries use total length as the measure of size classes. Danish and Scottish laboratories use 0.5 cm classes, whereas Norway uses one cm classes.

<u>Dissection and mounting</u>
In Denmark and Scotland one sagitta and in Norway both sagittae are dissected from each fish, with a maximum of 10 per size-class in Denmark, 20 - 40 per sample in Norway and 60 per sample in Scotland. All three laboratories dissect

frozen or newly thawed individuals. Otoliths are stored in groups by size class in Denmark and individually in Norway and Scotland. Before examination the standard procedure in Denmark is to mount the otoliths from each length group of a specific sample in a single row on a standard microscope slide (7.5 cm x 2.5 cm) with transparent nail varnish. The sagitta otoliths are mounted with sulcus side up and the surface uncovered by varnish. Some of the samples are read directly in their black plastic containers after being covered by alcohol. Scottish otoliths are read without mounting, and are covered by alcohol directly in their containers. Norwegian otolith pairs are mounted on a black back-ground covered by eukitt or nail varnish.

Otolith observation

Age determinations in all three countries are conducted using a dissection microscopes with magnifications from 16 - 40 times and reflected light. Care is taken to use a magnification which allows for a view of the entire otolith. Comparison among readers at the Danish laboratory is facilitated by a colour video-camera and monitor or parallel observation in a double dissection microscope.

Identification

Species identification and separation of otoliths accordingly is conducted routinely at the sampling stage. In all three countries individuals are determined to one of the groups *Ammodytes sp., H. lanceolatus, and G. semisquamatus*. Further separation into *A. marinus* and *A. tobianus* is conducted in field on Scottish samples, whereas this separation is determined from otolith morphology in Danish samples with an unknown accuracy.

Age determinations

Danish and Scottish readers make one age determination per individual fish with an occasional check, whereas Norwegian readers make two independent readings per individual. Routine ageing of sandeel otoliths is conducted by one reader in Scotland, two in Norway and several in Denmark. Information on area, date and size is known to all readers. Only Norwegian readers grade the ageing quality of individual otoliths. Validation of annual increments have only been conducted in Scotland, but no documentation from that exercise was available for the workshop. A short introduction to the ageing procedure is available in Denmark as well as in Scotland. Norwegian and Scottish data were retrievable at the individual level, whereas data from Danish samples only had size class affiliation, and otoliths by size groups were kept for reference.

Age determination criteria

The definition of age determination criteria seems to be rather subjective and not easily quantified. By direct interview most readers would agree to the following guidelines:

Translucent zones are interpreted as winter zones dependent on

1) their contrast to opaque zones
2) their width in relation to estimated age at formation
3) their position in a series of yearly increments with declining widths.

Further help when deciding on age is: fish size, size of otolith and date in relation to catch area. It was suggested that attention should be paid to the transparency of the outermost zonation in relation to time and first estimation of age class. Also the nucleus could contain information relevant for ageing.

Year- and age-class separation
All laboratories agreed on distinguishing year-classes according to expected hatching date with a separation date of 1st of January. Age-classes then follow hatch- and catch dates accordingly.

First otolith Intercalibration exercise

The intercalibration was organised by Erlend Moksness, IMR, Arendal (Norway). The Intercalibration was carried out during June - August 1995 and involved 7 readers from Denmark, Norway and UK (Scotland). A sample of otoliths from 355 sandeels were included in the exercise. The otoliths used were from 12 samples, originating from different areas (see figure 3.1), seasons, species and lengths of sandeels (see table 3.1). Otoliths from both *A. marinus* and *H. lanceolatus* between 7 and 29.5 cm were examined. The otoliths were either prepared as dry (sample 3 and 5; normally examined in alcohol) or mounted with eukitt or nail polish on black plastic trays (otoliths from 10 - 25 fish on each sample). Of the total sample (see Tab. 3.1 and Fig. 3.1), 184 were from the Shetland area (sampled in August), 90 from the Outer shoal (sampled in May), 31 from Karmøy and 50 from the Danish coast (sampled in April/May). Of the 7 readers only one had good experience of reading sandeel otoliths (Tab. 3.2), two had 3 to 5 years experience, while four had limited experience ageing sandeels.

The consistency in the estimated ages between readers and laboratories in the exercise was considered from calculations of mean age and the coefficient of variation (CV) for each sample and laboratory. Inter-reader bias was tested for each sample using a Wilcoxon signed ranks test.

Review work

The statistical analysis of results from the exercise were reviewed. From this review, effort was directed to an examination of samples with either low or high disagreement between readers. Otoliths that were either easy or difficult to interpret were selected in order to develop guidelines for the interpretation of increment structures. Photographs of some selected otoliths were produced for the report, giving both representative examples of typical otolith zone formation as well as secondary structures (i.e. false and split rings). Daily increment analysis was used to investigate suspected false and split ring structures (for details of preparation see section 6). Measurements of opaque and translucent zones were made using an eyepiece graticle, in order to provide some indication of the relative proportions of the two zones in a typical annual increment. The importance of having information on the time of the year, length of the fish and geographic region for assigning age were also reviewed.

Second intercalibration exercise

To test whether guidelines (see section 7) developed during the workshop reduced age reading differences between readers and laboratories, a second intercalibration exercise was conducted following the review described above. The geographical range in the first intercalibration exercise was considered to have been incomplete and consequently otoliths from the Danish coast and Dogger Bank area, together with samples from the Outer Shoal and Shetland were included in the second exercise (see tab. 3.3 and fig. 3.1).

All otolith terminology used in this report follows that given by Anon (1995b).

4. Results

4.1 The first otolith intercalibration exercise

A summary of the otoliths readings is given in Tables 4.1 and 4.3. Table 4.1 shows average age by plate no. determined by each of the laboratories. The agreement among laboratories was good in 7 out of the 12 samples. There were differences in the estimated age of sample 3 otoliths between laboratories. On average ages of the otoliths from sample 3 estimated by the Bergen laboratory were one year higher then those from the other two laboratories. In the other four samples (sample 11, 17, 18 and 21) the difference between the laboratories was between 0.5 to 1.0 year. The material did not indicate that any of the laboratories was consistently skewed compared to the others. Table 4.2 shows the average CV (%) by sample and each of the laboratories. The results indicate precision was high within two of the laboratories (CV < 15 %) and generally low in the third. As shown in table 4.3, 56 % of age readings by the third laboratory had a CV > 15 %, compared to 15.1 and 14.7 % respectively for the other two laboratories.

Table 4.4 shows results from a Wilcoxson signed ranks test comparing readers. The results indicate that in 61.2 % of the 240 combinations for all the samples, there was no significant bias between readers. In 25.4 % of the combinations there is a high probability of bias, and these were mainly found for age estimates from sample 1, 3, 5, 18 and 21.

4.2 The second otolith intercalibration exercise

From table 4.5 it appears, that there was only disagreement in sample 13 between the three laboratories, the age readings from Aberdeen being 0.6 years higher than the results from Bergen. In the other samples the differences between laboratories were between 0 and 0.3 years. The otoliths from sample 13 were from fish apparently larger and older than from other samples.

There was a high bias between readers for sample 4, i.e. in 15 out of the 21 combinations of the sample there was a significant bias between readers (table 4.8). The disagreement in age readings were related to the ageing of larger sandeels. For the other samples there was a low between reader bias. Sample 4 were from the autumn, and the other samples from the spring and summer.

The within laboratory CV listed in table 4.6 indicates a low level of precision in the Danish laboratory as was found in the first inter-calibration exercise.

The results from the second intercalibration exercise can be summarised as follows:

- The consistency in age estimates generally improved in the second intercalibration exercise compared to the first, indicating that the formulated protocol for age determination given in section 7 had been of some benefit.

- However, there still seems to be a certain degree of disagreement in the consistency of the age estimates between readers and laboratories for fish older than 1 year. Marginal annual increments in the otoliths of older individuals may be very narrow and there is a high probability that split rings may be present and for 1st year rings to be obscured by overgrowth.

- The improvements in inter-reader variation in age estimates of younger sandeels is demonstrated in table 4.7, where the consistency in age estimates between readers has been compared for the two intercalibration exercises. In the absence of reader D at the second exercise, the results of reader A, B, C, F, G and H were compared to reader E, because reader E had the closest agreement to the expert reader D during the first exercise. The improvement in consistency clearly appears for both 0- and 1-group sandeels.

5. Discussion

At the Workshop different techniques relateing to the handling and preparation of otoliths were discussed. The workshop concluded that the best results were achieved when the otoliths are cleaned properly and read immediately in ethanol. Good results could also be achieved with dried otoliths, and otoliths stored in ethanol or mounted in a resin.

Discrepancies and bias were experienced both between laboratories and individual readers. This was most pronounced with the oldest fish. A consensus age was reached on 107 of 355 otoliths. However, no one of these fish was more than 3 years-old. The readers in Bergen had some problems with the 0-group fish caught in August. These fish had started to lay down the first translucent zone and were estimated to be one-year-old. Another problem was the finding that some fish can lay down another opaque zone during the first summer/autumn because of resumed growth. This was discussed and it was agreed that the first true tranlucent zone is wide and easily distinguishable. It was also agreed that familiarity with area, time of sampling and size of the fish was required for a correct interpretation of the ring structure.

Listed below are comments and possible sources of bias in the first otolith reading exercise:

1. Problems encountered with otolith readings were related to experience, preparation of otoliths and "atypical" otolith structures.

2. Difficulties in resolving the narrow outermost increments of large otoliths made it difficult to assign an age to older fish (estimated age >4 years old).

3. Consideration of season proved to be very important in assigning an age, because the same pattern of zone formation was seen in different ages of fish. For example, an opaque and translucent zone from a fish sampled in August would indicate an 0-group fish whilst the same zone pattern in spring would indicate a 1-group fish.

4. In addition to date of capture, information on size and location of catch was also thought to be of use in estimating age. Nevertheless, individual differences in the time of onset of opaque and translucent zone formation was found to lead to further confusion.

5. Possible false and split rings occurred many of otoliths from *A. marinus* examined and were a major source of problems in assigning age. Grinding otoliths to examine otolith microstructure did appear useful in some cases (see section 7.3).

6. False rings also appeared to be a major problem in inferring the age of *Hyperoplus*. Further work is required to understand otolith structure, particularly with regard to the expected 1st zone structure.

6. Protocol for handling sandeel otoliths including preparation of 0-group otoliths for counting of daily increments

Isolation

Sagitta otoliths can be removed via an oblique cut through the dorsal surface of the head. Lapilli may also be obtained in this manner, although they are generally easier to locate after removing the operculum and cutting up through the basio-occipital pad. Any adhering tissue around the otolith can be removed with a 1% solution of sodium hypochlorite, although this is rarely necessary for fresh specimens.

The sagitta and lapilli are generally easy to see in larvae (< 10mm TL), although polarised light is sometimes useful to locate them. Larval otoliths can be excised using mounted needles, and in the case of larvae < 10mm TL the otoliths can be viewed directly.

Preparation for examination of annual increments

For annual increments, sagitta otoliths are generally viewed with a binocular microscope using reflected light against a black background. Transmitted light can elucidate more otolith structure but this method generally makes it more difficult to distinguish annual increments. Otoliths may be immersed in water, ethanol or clearing agents such as baby oil, glycerol or immersion oil. Permanent mounts may also be made with the use of resins. Due to the difficulty of viewing the narrow outer increments in some older specimens (>3 years old) and the presence of false annuli in some specimens it is occassionally beneficial to embed and grind otoliths before viewing (see below).

Preparation for otolith microstructural examination

The easiest form of preparation involves attachment to glass slides with an adhesive. Both cyanoacrylate adhesive (Bostick superglue) and methacrylate adhesive polymerised by UV light (Loctite Glass Bond) have been used. The latter provides a better attachment of the otolith to slides. However, the ability to detach sagittae is useful for larger specimens as grinding on both sides is often desirable. In the case of cyanoacrylate adhesive mounts, detachment is often possible after soaking in water. When only one side is ground the sagittae are attached with the *sulcus acusticus* facing the slide. When sagitta preparations are made for viewing daily increments in zonations laid down during late juvenile and adult growth grinding and polishing from both sides is often nessessary. In that case utilisation of thermo-plastic cement facilitates fast mounting and dismounting so the otolith may be flipped over several times to provide optimal focus on the critical microstructure. The sagitta can be ground with fine carborundrum powder (eg 1200 + 600 grit) or diamond pastes. A lapping wheel or other automated device is useful for grinding otoliths from juvenile sandeels. Sagittae from larvae (> 10 mm TL) and lapilli from all sizes of 0-group sandeels are best ground by hand. Hand polishing can be carried out with 1 μm diamond paste on a woven silk cloth. In both cases the otolith is periodically checked for signs of the core or yolk-sac resorption check (see below). The otoliths are then washed, dried and left to clear in a non-acidic immersion oil prior to examination.

Otolith microstructure feature

An example of otolith microstructure is shown in Plate 1. The high optical density of juvenile sagittae is a major problem and it is often difficult to get both the nuclear region and edge suitably ground. Increments are generally narrow (0.8-1.5 μm) close to the primordium and so require magnifications of x1000. Primary increments outside the nuclear region of 0.9 - 6.0 μm in width, have been recorded. 9-16 increments are generally formed by the stage of yolk-sac depletion. A check coinciding with the time of yolk-sac absorption is usually visible. The hatching check is also evident in the sagittae of larger larvae and juveniles.

Sagitta shape differs between life-history stages, being circular in larvae and oval in juveniles and adults. The transformation in sagitta shape occurs over the length range 35-55 mm TL and is associated with a change in sagitta growth from accretion around a central primordium, to the formation and accretion around two secondary growth centres (also known as accessory primordia or peripheral nuclei). The posterior or post-rostral growth centre is only seen in juvenile specimens, indicating that the appearance of this secondary growth centre is related to stage of development. Accretion around the secondary growth centres results in the overgrowth of material laid down during the larval phase and a marked increase in the opacity of the sagitta.

7. Protocol for age determination of otoliths

7.1 Age determination criteria

An annual increment is composed of an opaque and a translucent zone. Seasonal changes in the appearance of opaque and translucent zones for a given year-class are given in Table 7.1. Examples of 1- and 2-group sandeel otoliths from spring are shown in Plate 2 and 3, respectively. Examples of 0-, 1-, and 3-group sandeel in autumn are shown in Plates 4 to 6. The date of birth is assumed to be 1 January and the fish is assigned to a year class on this basis.

7.2 Other available information

Other information that might be available about the fish will be the total length, sex and maturity stage. The length information may influence the decision of the reader when assigning an age. However, even without information an experienced otolith reader can infer the approximate length of the fish from the diameter of the otolith.

7.3 False and split rings

Split rings

Split rings appear as two or more translucent zonations separated by narrow opaque zone(s) which do not conform with the regular sequence of annual increments. The width of a typical first translucent zone is 8 - 14% of the total annual increment. The width of a cluster of split rings is often wider than that of a typical translucent zone (see Plate 7a and b). The individual zonations in a split ring cluster may differ in appearance both with regard to width and transparency. An analysis of otolith microstructure in split ring clusters revealed that primary (presumed daily) increments were evident in both translucent and opaque zones. It has not been shown that an opaque zone and a split ring cluster together constitute an annual increment.

False rings

False rings appear as one or more narrow translucent zonation(s) in the opaque zone of the 1st year (Plate 8). These structures are common in *H. lanceolatus*. Features that may distingquish false rings from the translucent zone of an annual increment are that the width of the preceding opaque is less than that expected for a complete opaque zone, or that the width of the following opaque zonation is less than that expected for a complete 2nd year opaque zone. Daily increments continue with almost unchanged width between the opaque zonation and translucent zonation of a false ring (Plate 9).

Obscured annual increments
The translucent zones of the first and possibly second year of the otolith may become hidden by overgrowth of otolith material. In such cases the translucent zone may be only partly visible from either the distal of the lateral side of the sagitta depending on age and otolith growth pattern. Grinding and polishing may improve readability by removing some of the recently accreted material.

8. Summary and conclusions

The present Workshop on Sandeel Otolith Analysis was established as a result of a recommendation adopted by the 1994 Statutory Meeting (Anon., 1995a). According to the terms of reference a general evaluation of the biology of the lesser sandeel *A. marinus* was given. Two different age determination exercises were held according to the methods described by the Workshop on Sampling Strategies for Age and Maturity; one in front of the Workshop, the other one during the Workshop. Protocols describing handling of otoliths, preparation of otoliths and age determination were made during the Workshop. Secondary ring structures were studied and discussed and recommendations concerning age determination were given. Discrepancies and bias were experienced both between laboratories and individual readers in the age determination exercises. It was discovered that it was impossible to produce guidelines giving sufficient precision in the readings. This was mainly caused by variations in the pattern of increment deposition. However, the precision of the estimates did increase in the second exercise due to the discussions from the first exercise carried out during the Workshop.

9. Recommendations

Despite a significant improvement in the consistency of age estimates for 0- and 1-group sandeel otoliths, it was not possible to provide a sufficiently detailed key to identifying secondary structures in older individuals. Based on this experience the workshop recommends that:

- For consistency among readers reference should be made to the protocol in section 7 in this report.

- More work is needed to characterise the morphology of otolith growth structures and the geographic and seasonal variations in these from the North Sea and adjacent areas. Special attention should be paid to the statistical variation in transparency of the marginal zonation with date, location, otolith size and apparent age.

- Further research should be directed to validating procedures for different structural appearances of annual increments.

- Reference collections for use in future inter-calibration exercises and for exchange between laboratories should include information on species, sampling time, area and length of the fish.

- Sandeel otolith exchanges between laboratories should be conducted regularly.

- A reading form should be constructed along with a short key to different secondary structures that should be recorded in connection with each individual age determination. The interpretation of different structures at different levels of age could identify error distributions by age and help design procedures for correction or elimination of questionable otoliths for routine age determination.

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11. Appendix

Tables

Table 3.1. Information on each of the sample of sandeel otoliths included in the Intercalibration exercise.

Sample.	No. otoliths	Species	Fish size (cm)	Date	Location	Preparation
1	25	A. marinus	11,0 - 21,5	1-6/5-94	Outer shoal	Eukitt
2	25	A. marinus	13,5 - 29,5	1-6/5-94	Outer shoal	Eukitt
3	94	A. marinus	7,0 - 18,0	7-8-94	Shetland	Dry
5	90	A. marinus	11,0 - 24,0	6-8-93	Shetland	Dry
7	10	A. marinus	13,0 - 16,0	19/4	Outer shoal	Eukitt
8	10	A. marinus	13,0 - 15,0	19/4	Outer shoal	Eukitt
11	10	A. marinus	15,0 - 20,0	24/4	Outer shoal	Eukitt
12	10	A. marinus	14,0 - 20,0	24/4	Outer shoal	Eukitt
16	25	A. marinus	15 - 20	April	Karmøy	Eukitt
17	6	H. lanceolatus	20 - 30	April	Karmøy	Eukitt
18	25	A. marinus	8,5 - 18,0	May-95	Danish coast	Eukitt
21	25	H. lanceolatus	17,5 - 28,0	May-95	Danish coast	Eukitt

Table 3.2. Reader experience indicated for each reader by number of years of experience and approximately number of otoliths examined.

Reader	Year	Otoliths
A	0	800
B	0	700
C	0	500
D	15	> 10.000
E	5	2.000
F	3	2.000
G	0	500
H	0	0

Table 3.3. Information on each of the Plates with sandeel otoliths included at the Workshop exercise.

Sample	No. otoliths	Species	Fish size (cm)	Date	Location	Preparation
4	50	A. marinus	7.0 - 21.5	4-8-94	Shetland	Dry
10	10	A. marinus	9.0 - 20.0	19-4	Outer shoal	Eukitt
13	10	A. marinus	15.0 - 21.0	24-3	Outer shoal	Eukitt
19	25	A. marinus	9.0 - 20.0	May-95	Danish coast	Eukitt
Dogger	75	A. marinus	12.5 - 18.5	May/June	Dogger	Nail vanish

Table 4.1. Range and average (Av) of age determination and by laboratories in the total material (Total).

Sample	No. Otoliths	Total Range	Av	Denmark Range	Av	Scotland Range	Av	Norway Range	Av
1	25	0-7	1,96	1-7	2,09	0-3	1,48	1-3	2,00
2	25	1-6	2,08	1-5	2,10	1-5	2,12	1-4	2,04
3	94	0-5	1,09	0-5	0,82	0-4	0,85	1-4	1,74
5	90	0-6	2,26	0-6	2,15	1-6	2,37	2-5	2,21
7	10	0-3	1,84	0-3	1,80	1-3	1,95	1-2	1,80
8	10	1-3	1,89	1-3	1,73	2	2,00	2	2,00
11	10	2-5	3,01	2-5	2,90	2-5	3,45	2-3	2,75
12	10	1-5	2,51	1-4	2,50	2-5	2,55	2-4	2,50
16	25	1-9	4,04	1-9	4,01	1-9	4,18	1-9	4,38
17	6	2-8	4,83	2-7	4,33	3-8	4,92	4-8	5,50
18	25	1-4	2,15	1-4	2,37	1-4	1,84	1-4	2,12
21	25	1-6	3,26	1-6	2,71	2-6	3,48	2-6	3,88

Table 4.2. Average calculated CV in the total material (Total) and group by laboratories.

Plate code	Total	Denmark	Scotland	Norway
1	0,29	0,32	0,13	0,04
2	0,12	0,10	0,07	0,02
3	0,83	0,23	0,10	0,04
5	0,09	0,09	0,05	0,02
7	0,21	0,31	0,03	0,09
8	0,22	0,38	0	0
11	0,13	0,07	0,03	0,03
12	0,13	0,16	0,02	0
16	0,06	0,04	0,04	0,07
17	0,19	0,17	0,11	0,08
18	0,33	0,41	0,04	0,09
21	0,28	0,36	0,07	0,12

Table 4.3. Proportion of otoliths on each plate with a CV > 15%.

Plate code	Total %	Denmark %	Scotland %	Norway %
1	60,0	60,0	20,5	8,0
2	44,0	24,0	16,0	8,0
3	82,8	16,0	11,5	12,5
5	38,0	22,8	13,9	6,3
7	90,0	80,0	10,0	20,0
8	100,0	100,0	0,0	0,0
11	60,0	30,0	10,0	10,0
12	50,0	60,0	10,0	0,0
16	16,0	12,0	4,0	15,0
17	66,7	66,7	50,0	16,7
18	92,0	100,0	8,0	24,0
21	96,0	100,0	28,0	56,0
Average	66,3	56,0	15,1	14,7
SD	26,4	34,2	13,2	14,9

Table 4.4. Inter-reader bias test (Wilcoxon; Anon, 1994). - : no sign of bias ($p > 0.05$); * : possibility of bias ($0.01 < p < 0.05$); ** : certainty of bias ($p < 0.01$).

Sample 1

	A	B	C	D	E	F	G
A							
B	*						
B	*	**					
C	*	-	**				
D	-	-	**	*			
E	-	**	*	**	**		
F	-	**	*	**	**	-	

Sample 2

	A	B	C	D	E	F	G
A							
B	-						
B	*	*					
C	-	-	-				
D	*	*	-	-			
E	-	-	-	-	-		
F	-	-	*	-	-	-	

Sample 3

	A	B	C	D	E	F	G
A							
B	-						
B							
C	-	**					
D	*	-		*			
E	**	**		**	**		
F	**	**		**	**	**	

Sample 5

	A	B	C	D	E	F	G
A							
B							
B	**						
C	**		**				
D	-		**	-			
E	-		**	**	-		
F	-		**	**	-	-	

Sample 7

	A	B	C	D	E	F	G
A							
B	-						
B	-	-					
C	-	-	-				
D	-	-	-	-			
E	-	-	-	-	-		
F	-	-	-	-	-	-	

Sample 8

	A	B	C	D	E	F	G
A							
B	**						
B	**	-					
C	**	-	-				
D	**	-	-	-			
E	**	-	-	-	-		
F	**	-	-	-	-	-	

Sample 11

	A	B	C	D	E	F	G
A							
B	-						
B	-	-					
C	*	*	-				
D	*	*	-	-			
E	-	-	-	-	*		
F	-	-	-	-	*	-	

Sample 12

	A	B	C	D	E	F	G
A							
B	*						
B	*	-					
C	*	-	-				
D	-	-	-	-			
E	-	-	-	-	-		
F	-	-	-	-	-	-	

Sample 16

	A	B	C	D	E	F	G
A							
B	-						
B	**	**					
C	*	**	-				
D	*	*	-	-			
E	*	**	-	-	-		
F	-	-	*	*	-	*	

Sample 17

	A	B	C	D	E	F	G
A							
B	-						
B	-	*					
C	-	*	-				
D	-	-	-	-			
E	*	*	-	*	-		
F	-	-	-	-	-	-	

Sample 18

	A	B	C	D	E	F	G
A							
B	-						
B	**	**					
C	-	-	**				
D	-	-	**	-			
E	-	**	**	**	**		
F	-	*	**	-	*	-	

Sample 21

	A	B	C	D	E	F	G
A							
B	**						
B	**	**					
C	**	-	**				
D	**	-	**	-			
E	**	**	**	**	**		
F	**	-	**	-	-	**	

Table 4.5. Range and average (Av) of age determination in the total material (Total) and group by laboratories.

	Total		Denmark		Scotland		Norway	
Sample	Range	Av	Range	Av	Range	Av	Range	Av
4	0 -7	1.6	1 - 5	1.4	0 - 7	1.7	0 - 5	1.7
10	1 - 4	1.6	1 - 4	1.6	1 - 4	1.7	1 - 4	1.6
13	2 - 4	2.7	2 - 4	2.8	2 - 4	3.1	2 - 4	2.5
19	1 - 3	1.7	1 - 3	1.6	1 - 3	1.7	1 - 3	1.9
Dogger	1 - 3	1.6	1 - 3	1.5	1 - 3	1.5	1 - 2	1.5

Table 4.6. Average calculated CV in the total material (Total) and group by laboratories.

Plate code	Total	Denmark	Norway
4	0.16	0.17	0.03
10	0.13	0.02	0.00
13	0.14	0.07	0.08
19	0.14	0.11	0.06
Dogger	0.29	0.07	0.05

Table 4.7. Percentage estimated age of otoliths which has been allocated as = and 1 year old respectively. The table includes age estimates from reader: A, B, E, F and G.

	Age 0		Age 1	
	First calibration	Second calibration	First calibration	Second calibration
Tot. otoliths	33	20	44	71
Tot. Obs	165	100	220	426
Estmated age				
0	20.0 %	100 %	1.8 %	0 % %
1	39.4 %	0 %	77.0 %	94.8 %
2	40.6 %	0 %	52.7 %	4.5 %
3	0.6 %	0 %	1.8 %	0.7 %

Table 4.8. Inter-reader bias test (Wilcoxon; Anon, 1994). - : no sign of bias (p > 0.05); * : possibility of bias (0.01 < p < 0.05); ** : certainty of bias (p < 0.01).

Sample 4

	A	B	C	E	F	G	H
A							
B	**						
C	-	**					
E	**	-	**				
F	**	-	**	-			
G	**	-	**	-	*		
H	**	**	**	**	**	**	

Sample 10

	A	B	C	E	F	G	H
A							
B	-						
C	-	-					
E	-	-	-				
F	-	-	-	-			
G	-	-	-	-	-		
H	-	-	-	-	-	-	

Sample 13

	A	B	C	E	F	G	H
A							
B	-						
C	-	-					
E	-	-	-				
F	-	-	-	*			
G	-	-	*	*	-		
H	-	*	-	*	-	-	

Sample 19

	A	B	C	E	F	G	H
A							
B	-						
C	-	-					
E	-	-	-				
F	-	*	-	-			
G	**	**	**	*	*		
H	-	-	-	-	-	**	

Sample Dogger

	A	B	C	E	F	G	H
A							
B	-						
C	*	*					
E	-	-	-				
F	-	-	-	-			
G	-	-	-	-	-		
H	**	**	**	**	**	**	

Table 7.1. Seasonal changes in the appearence of opaque and tranlucent zones for a given year-class. Key to season: early spring = March; late spring = April-May; summer = June-July; autumn = August ->.

Season	year-class	Outermost zone
early spring	0-group	N.A.
	1-group	broad translucent zone
	2-group	N.A.
	3-group	N.A.
late spring	0-group	N.A.
	1-group	broad translucent zone or partial 2nd opaque zone
	2-group	2nd translucent zone
	3-group	3rd translucent zone
summer	0-group	opaque zone
	1-group	2nd opaque zone
	2-group	partial 3rd opaque zone
	3-group	partial 4th opaque zone
autumn	0-group	opaque zone or partial translucent zone
	1-group	complete 2nd opaque zone or partial 2nd translucent zone
	2-group	complete 3rd opaque zone or partial 3rd translucent zone
	3-group	complete 4th opaque zone or partial 4th translucent zone

Figures

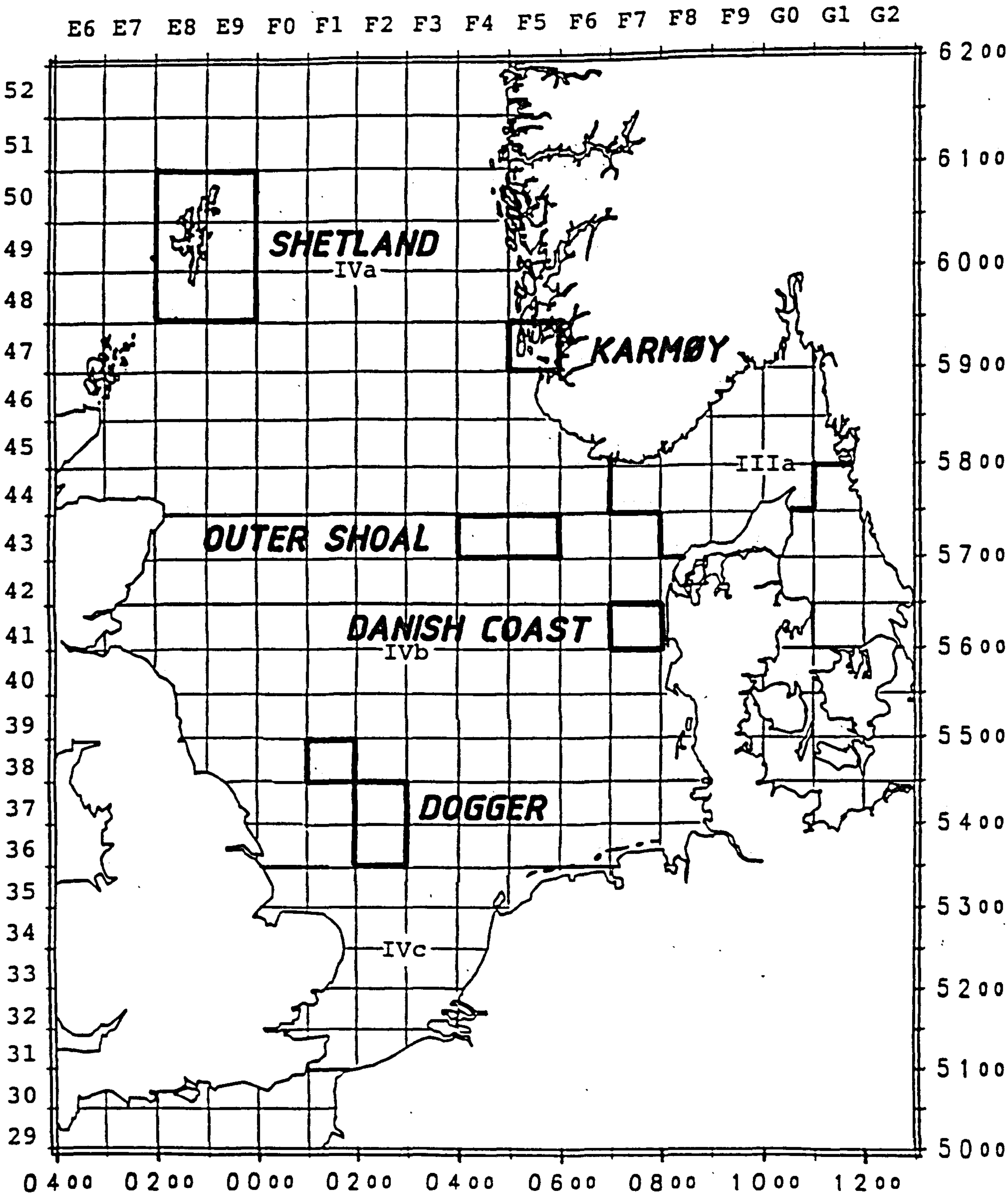


Figure 3.1. Map showing location of samples used in the two inter-calibration exercises.

Plates

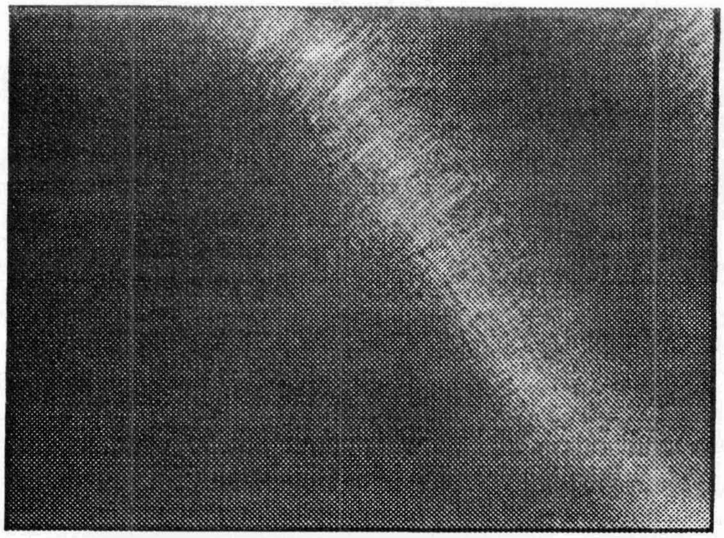


Plate 1 Otolith daily increments seen in the translucent zonation of a false ring. Note the constant increment width of about 5,5μm between the opaque and the translucent zonation.
Scale: 1cm = 29 μm

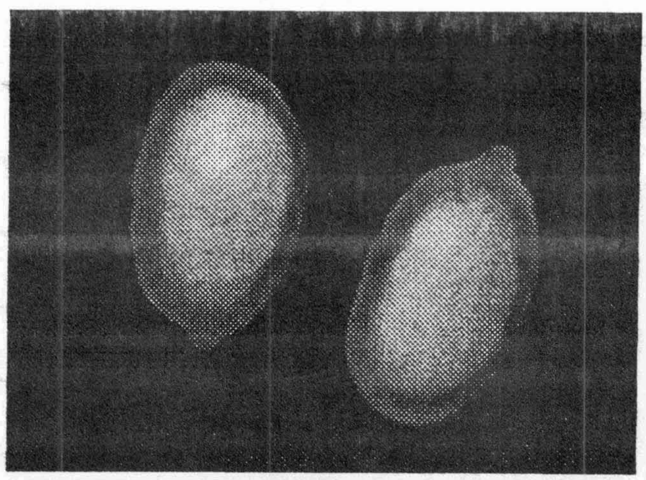


Plate 2 Otolith from a 1-group A. marinus caught in May on the Outer Shoal. Note that the marginal zone is opaque.
Scale: 1cm = 0.4mm

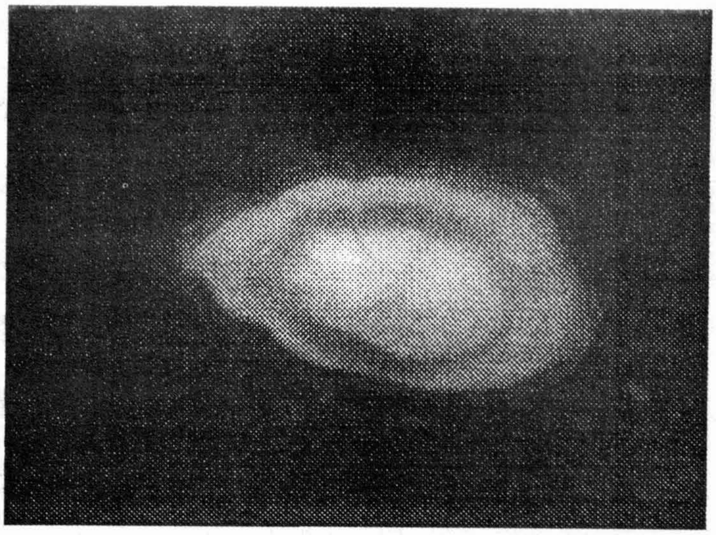


Plate 3 Otolith from 2-group A. marinus caught in May on the Outer Shoal. Note that the marginal zone is translucent.
Scale: 1cm = 0.4mm

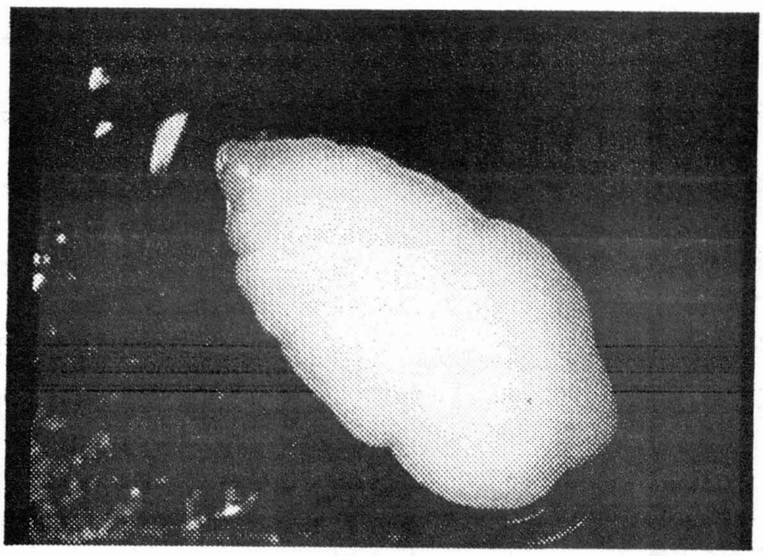


Plate 4 Otolith from 0-group A. marinus caught off Shetland in August.
Scale: 1cm = 0.2mm

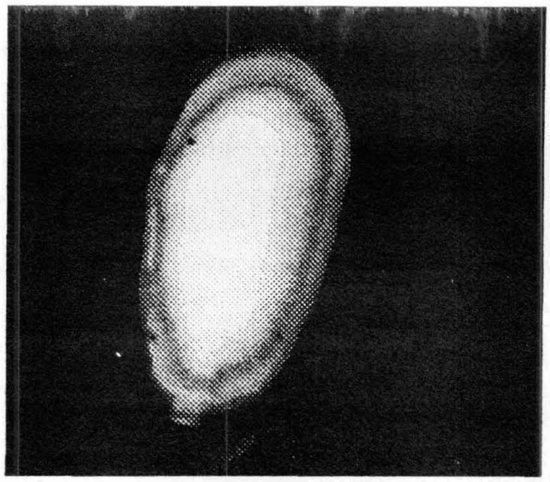


Plate 5 Otolith from a 1-group A. marinus caught off Shetland in August. Note the marginal translucent zone.
Scale: 1cm = 0.4mm

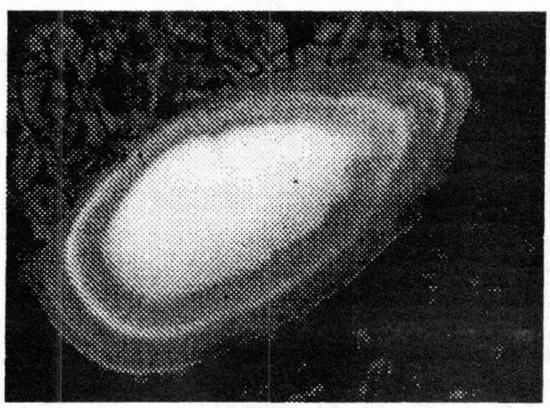
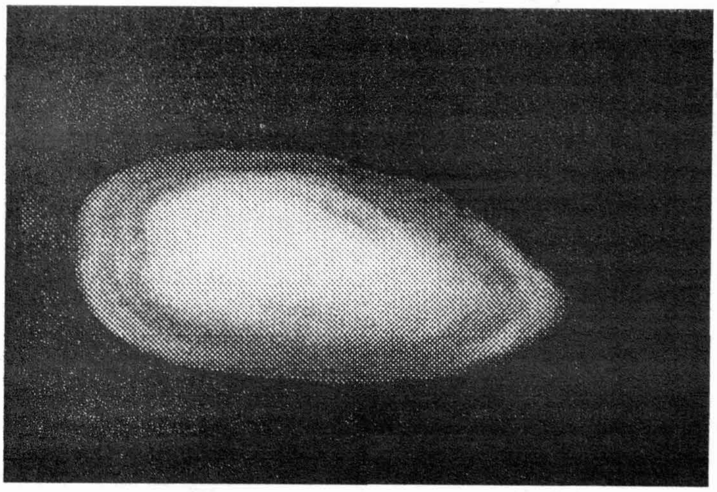
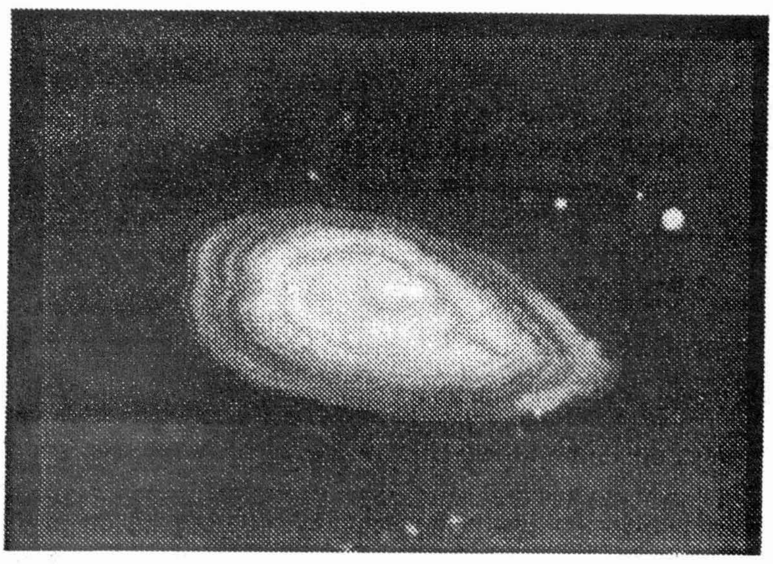


Plate 6 Otolith from 3-group A. marinus caught at Shetland in August.
Scale: 1cm = 0.4mm



a



b

Plate 7a & b Otolith of A. marinus caught at Shetland with suspected split ring (post rostrum) and a false ring.
a shows unground otolith. Scale: 1cm = 0.38mm
b shows sagitta after grinding. Scale: 1cm = 0.4mm

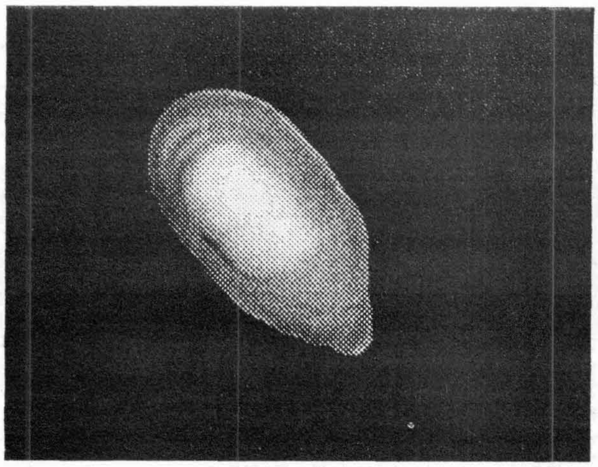


Plate 8 Otolith of A. marinus caught on the Outer Shoal in May showing suspected false rings.
Scale: 1cm = 0.4mm

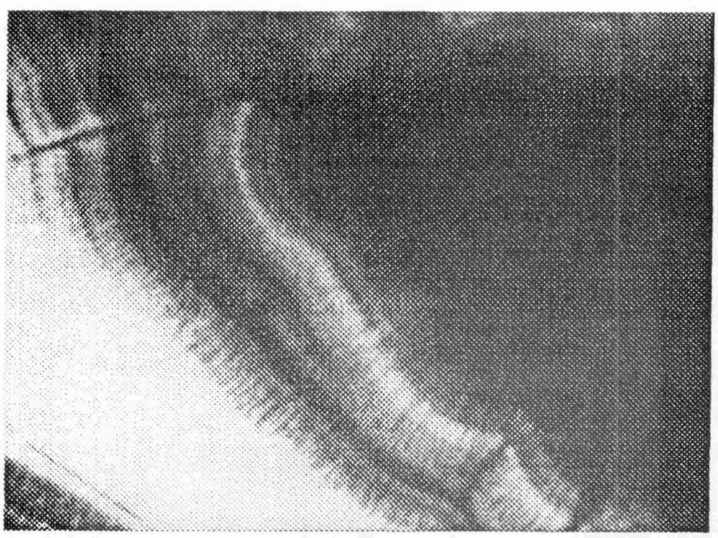


Plate 9 Otolith microstructure of suspected false ring (see also plate 1).
Scale: 1cm = 70 µm