Intercalibration of age readings of ling (Molva molva L.) blue ling (Molva dipterygia Pennant, 1784) and tusk (Brosme brosme L.)

O. A. Bergstad, J. V. Magnusson, J. M. Magnusson, N.-R. Hareide, and J. Reinert

Introduction

The stocks of ling Molva molva (L.), blue ling Molva dipterygia (Pennant, 1784) and tusk Brosme brosme (Ascanius, 1772) in the north-eastern Atlantic support significant coastal and offshore bank and slope fisheries in several countries (ICES, 1996). The knowledge of the population biology, stock structure and stock size changes in response to exploitation of the three species has, however, been very limited (ICES, 1996).

Early data on the occurrence of eggs and larvae (Schmidt, 1909) showed the widespread spawning of the three species, and pioneer studies of size composition, growth and distribution by Molander (1956) and Joenoes (1961) provided the first insights into their biology and ecology in north-east Atlantic waters. More recent studies and monitoring have supplemented these early studies (e.g. Magnusson, 1978, 1979, 1980, 1981, 1982a,b, 1983a,b; Engås, 1983; Ehrich and Reinsch, 1985; M oguedet, 1985, 1987; Thomas, 1987; Grotnes and Hareide, 1989), and provided time-series of length measurements and abundance in routine surveys and new information on biology. Because of methodological shortcomings, in particular accurate and precise age determination methods, many results have been of limited value.

In 1992, Iceland, the Faroes and Norway initiated a project to improve knowledge on biology of the different species, focusing specifically on age determination to form a basis for future stock assessments and studies of biology. As for many other deep-water species (Bergstad, 1995), both validation of age readings and intercalibration among readers was needed. This paper summarizes efforts during the period 1993–1996 to intercalibrate age determinations by sagittal otoliths and first attempts to validate the age readings of juveniles.

Material and methods

Ling

Molander (1956) described methods for reading whole ling otoliths, and his methods were used in later studies...
of this species (Bergstad, 1991), and in this project. The otoliths were submerged in water or glycerol and viewed sulcus-side down by bright transmitted light or by reflected light against a black background. Few otoliths from ling of total length (TL) greater than around 90 cm could be read whole. Breaking and viewing of the broken surface with illumination from the side and shading of the surface was found to be a preferred technique and a good alternative to sectioning. The otoliths could be kept dry or in ethanol or water after collection. If kept dry, soaking in water was necessary to re-expose the annuli. A particular difficulty when reading whole ling otoliths was the definition of the first fast-growth zone, i.e. the nucleus and the opaque area deposited during the 0-group stage. This area of the otolith had very variable size and sometimes seemed very small compared with the subsequent opaque zones, probably because the spawning time is prolonged. Three intercalibration exercises were run for ling. The otoliths came from fish caught west of Britain, off western Norway and in the Skagerrak.

Blue ling

Previous attempts to age blue ling involved reading annuli from broken surfaces (Magnusson, 1982a,b) or thin sections (Engås, 1983; Ehrich and Reinsch, 1985; Thomas, 1987; Bergstad, 1991). In this study, 0.2-0.3 mm thick transverse sections were produced by a procedure developed by Bedford (1983), or by a double-blade ISOMET saw as described by McCurdy (1985). Mounting of the sections in clear resin seemed to be a good alternative to using black resin, especially if the sections were examined by a compound microscope.

The interpretation of the first few annuli was a particular problem with blue ling. The first fast-growth zone (opaque zone) appeared many times to be split into fairly distinct sub-zones. To obtain consistent readings it was important to consider the relative width of successive opaque zones. Few small blue ling occur in samples from most fishing grounds, and only a limited number of juveniles from Iceland was used in intercalibrations. Attempts were made to validate the age readings for the first few age-groups by considering the progression of modes in length-frequency distributions from annual Icelandic groundfish surveys.

Tusk

Otoliths from tusk of TL<70 cm were viewed whole submerged in glycerol sulcus-side up, using reflected light against a black background. For specimens of TL>70 cm transverse sections were produced by the double-blade ISOMET saw and mounted in clear plastic on microscope slides. Otolith collections from Icelandic and Norwegian waters were used in a series of intercalibrations. Length-at-age data for Icelandic juveniles were related to modes in length distributions from groundfish surveys.

Results

Ling

An exchange of 50 ling otoliths in 1994 showed that Iceland tended to estimate higher ages than others. However, when discussing the interpretations and considering Molander's (1956) results, it was easy to reach agreement on a common most probable interpretation of the annuli. This was confirmed when new independent readings of 20 ling otoliths were made (Table 1) and in 1996 by a new test based on a set of 93 otoliths (Fig. 1). The inconsistencies among readers were minor, and the precision of the age estimates was considered acceptable. Comparisons of ages read from whole and broken otoliths showed that there were no systematic differences (Fig. 2). The size range that could be aged was significantly extended by using broken otoliths.

Blue ling

Icelandic length-frequency distributions from groundfish surveys conducted every year in March showed modes among the smallest fish that probably represent

<table>
<thead>
<tr>
<th>Fish no.</th>
<th>Icel.</th>
<th>M F 1</th>
<th>M F 2</th>
<th>IMRF</th>
<th>Faroes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

MF = Møre Research (two readers), IMRF = Institute of Marine Research, Flødevigen Mar. Res. Station.
age-groups (Fig. 3). Age readings of transverse otolith sections from fish of TL <60 cm indicated that the two first identifiable modes were most probably 1- and 2-group. The 1-group fish were in March mostly less than 20 cm, the 2-group very variable between 20 and 40 cm. Using these observations as guidelines, it was possible to achieve reasonable consistency among readers but only for otoliths from juveniles up to
3–4 years old. A very high proportion of the otoliths from larger fish were regarded as unreadable, and no attempt was made to intercalibrate readings for the entire age-range.

**Tusk**

The interpretation problems were greater for tusk than ling, but not as grave as for blue ling. The opaque fast-growth zones were seldom homogeneous but appeared as bands split by thin but sometimes distinct hyaline zones easily misinterpreted as annuli. The nuclear region of the otolith showed variable size.

The interpretation differences were obvious in the first intercalibration (Fig. 4). The results improved after consensus on a common interpretation principle was reached and in a subsequent exchange (Fig. 5).

---

**Figure 2.** Frequency of differences between readings, by the same reader, of whole and broken ling otoliths from the same specimens. \( n = \text{number of specimens examined.} \)

---

**Figure 3.** Length distributions of blue ling <60 cm caught by bottom trawl on the Icelandic groundfish surveys in March every year.
Intercalibration of age readings

Figure 4. Comparisons of age readings of 49 tusk otoliths in 1994. The histograms show the difference between readings by two readers. p-values are probabilities from pairwise Wilcoxon Rank Sum tests. Length range: 41-74 cm. Reader codes as in Figure 2.

differences between readers were 1-3 zones of fish 7-15 years old and there was only limited bias. In some cases differences were much larger, which indicated more serious interpretation problems, or that the otoliths were of poor quality and should have been considered unreadable.

To test the stability with time, the same set of otoliths were re-marked and exchanged again after about
In most tusk samples a relatively high fraction of the fish in the length range 40–70 cm had otoliths that were considered unreadable (Figs 5 and 6), and only very few bigger fish could be aged with confidence.

6 months. There was an overall improvement in the consistency and no inter-reader bias (Fig. 6). This indicated that a common and reasonably stable interpretation had been achieved.

Figure 5. Comparisons of age readings of 75 tusk otoliths in 1995. Histograms show the difference between readings by two readers. p-values are probabilities from pairwise Wilcoxon Rank Sum tests. Length range: 37–82 cm. Cases where one or more readers had not recorded the age were excluded prior to the analysis (n=13). Reader codes as in Figure 2.
Analyses of Icelandic length distributions of tusk from March groundfish surveys (Fig. 7) and corresponding age-readings of specimens less than 40 cm TL, showed that overall, there was very good correspondence between the modes and the mean length of successive age-groups obtained by otolith ageing in the age-range 2–4 years. The first mode of the length distributions at a length around 15 cm represented 2-group

Figure 6. Comparisons of age readings of 92 tusk otoliths in 1996. The histograms show the difference between two readers. p-values are probabilities from pairwise Wilcoxon Rank Sum tests. Length range: 11–69 cm. Cases where one or more readers had not recorded the age were excluded prior to the analysis (n=41). Reader codes as in Figure 2.
and the 1-group fish were probably only 7–8 cm in March.

Discussion

The aims of this work were to develop common age determination methods and to test and improve the precision of age readings. Most of the effort was focused on tusk and ling, the two species that are most important in the Nordic fisheries. For small and medium-sized ling and tusk, relatively simple techniques proved successful and provided reasonably precise age readings. For large fish (i.e. ling > 90 cm and tusk > 70 cm) no fully satisfactory techniques were found. Some of the big ling otoliths could be read by the “breaking and shading” technique used for some other gadoids (e.g. Williams and Bedford, 1974). In the major fishing areas rather few of the largest fish occur in the catches, hence the lack of a good age determination for the very largest specimens may not become a major obstacle in future applications.

Because the blue ling otoliths were much less transparent than those from ling and tusk, sectioning seemed necessary, even for small fish (see also Engås, 1983; Thomas, 1987). However, on the thin transverse sections it was usually difficult to define consistently the annuli from the first 5–10 years. Only for the very small fish was it possible to define common patterns that were regarded as annuli. After a certain size the otolith seems primarily to grow by deposition of material on the proximal and distal surfaces (Engås, 1983; Bergstad, 1991) and thin growth zones, which may be annuli appear on the proximal sides. Similar structures were previously
described for e.g. Pacific sablefish (Anoplopoma fimbria) (Beamish and Chilton, 1982) and roundnose grenadier (Coryphaenoides rupestris) (Bergstad, 1990, 1995).

For both ling and tusk the inter-reader precision was improved over the course of the exercises. A problem was that inconsistencies tended to arise because the technicians concerned read ling and tusk otoliths infrequently. It seemed unlikely, however, that the precision could be enhanced much further. The level of precision obtained in our exercises is probably acceptable for future studies of many aspects of population biology, e.g. age distributions, growth, age-at-maturation, fecundity-at-age etc., and possibly for age-based assessment procedures.

In relation to establishing routine age determination for stock assessments, it is a problem that a relatively high proportion of the otoliths may be discarded as unreadable, i.e. as many as 20–30% of the tusk otoliths. Sample sizes will have to be large in order both to cover the full age-range of the species and take the probable discarding into account.

This work has not included validation of the otolith ages for the entire age range. This is an important task for the future. The structures assumed to represent annuli, at least in ling and tusk, look similar to those found in other gadoids and other fish, for which greater ages for the entire age range. This is an important task for discarding into account.

Acknowledgements

We are grateful to A. Buner, G. Garnes, A. Henriksen, K. Hansen, S. Rosseland, and T. Vidarsson for patient reading of many otoliths, and to Ø. Paulsen and S. E. Enersten at IMR Flødevigen for editing of some of the figures for the paper. The work was supported financially by grants from The Nordic Council of Ministers (Project No. 66.04.11.00) to all the three countries, and from The Norwegian Research Council to Norway (Project No. 104759/110).

References

Magnusson, J. V. 1981. Icelandic investigations on Blue Ling (Molva dypterygia) and Ling (Molva molva) in 1981. ICES Annales Biologiques, 38: 132-133.
Magnusson, J. V. 1983a. Icelandic investigations on blue ling (Molva dypterygia) and ling (Molva molva) in 1983. ICES Annales Biologiques, 40: 116-117.


Thomas, R. 1987. Biological investigations on the blue ling (Molva dypterygia (Pennant 1784 after O. F. Müller 1776)), in the areas of the Faroe Islands and to the west of the Shetland Islands. Archiv für die Fischereiwissenschat, 38(1/2): 9–34.