Report from

The Herring Age Reading Workshop

in the Archipelago Research Institute,
the island of Seili, Finland

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Content:

1. Introduction

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1.2 The objects of the workshop

As a part of the process of herring stock assessment, age determinations of herring are conducted in several laboratories in Atlantic and Baltic coastal areas. Age determination errors have been identified as one possible factor reducing the accuracy of stock assessment.

The aim of the workshop was to identify present problems in herring age determination, improve the accuracy and precision of age determinations and spread information of the methods and procedures used in different ageing laboratories working with herring.

In the workshop, two groups of age determination laboratories that have had co-operation inside each group before, those at the Atlantic coastal areas and those at Baltic Sea coastal areas were working together. The common meeting benefited both groups by changing experience of different practices and spreading knowledge of herring growth and otoliths in different conditions from those populations and areas each one was familiar with.

Before the workshop, four different samples of otoliths were circulated among different laboratories to assess the precision of age readers. Preliminary results from the circulation were presented in the workshop and discussed together.

EU data collection programme funded the participation of two members per EU member country.

2. Presentations

In the workshop, following presentations were given:

Willie McCurdy: Age determination of herring in Atlantic stocks.

Jari Raitaniemi: Ageing of slowly grown herring: experience from sawing and staining technique compared with ageing from whole otoliths.

Jan Eklund: The ageing of Bothnian Sea herring from whole otoliths and otolith slices

Georgs Kornilovs: Work of Baltic Herring Age Reading Study Group (BHARSG) 2001-2005.

Jari Raitaniemi: Presentation of results from otolith exchange and discussion.

Georgs Kornilovs: Age determination alternatives in Baltic herring from the Gulf of Riga.

3. The interpretation of otolith structures

Otoliths were examined together by projecting their images on the wall with data projectors from two microscopes: one used for examining whole otoliths and one for examining thin slices.

There were two main groups of otoliths examined:

1) Otoliths that had been included in the exchange of otolith samples and thus had been read by a number of readers

In the otolith exchange, the four samples circulated were from the Netherlands (2 samples with 25 otoliths each), Norway (50 otoliths), and Poland (50 otoliths). Altogether eight readers were able to participate in the exchange.

Main results from the exchange:
There were clear differences in the precision between different samples. In the easiest sample from the Netherlands (**NL 241001**), all readers determined 80–100% of the otoliths identically to the mode. In the other Dutch sample (**NL 241003**), the readers aged 56–92% of the otoliths identically to the mode. Four of the eight readers aged more than 80% of the otoliths similarly to the mode. Possibly because of the small number of the fish, 25 specimens, only in one case the result was statistically different from the mode (Wilcoxon's signed ranks test, 56% aged similarly, p = 0.001). Comparing the results of different readers to the Dutch readers, who were most experienced with the material, did not improve precision. However, the Dutch readers were closer to each other than to the others.

With the **Norwegian sample**, the interpretation of results was more complicated (North Sea herring serial no 60028): The age estimates of different readers were in two groups so that it was possible to select either lower mode or higher mode (equal number of two age values determined in a number of specimens). Most, but not all readers, had their estimates closer to the lower mode values, i.e. 12–92% of the determinations were identical. When one year was added to the determined age of two readers, who had written down the age of fish as year class, their numbers of identical ages with the mode value rose from 12% and 20% to 80% in both cases. When comparing the age estimates to the most experienced reader with this population, the result improved again a little, being 82–94% of identical estimates with the exception of one reader with 38% of identical age estimates with the reference reader. Mostly the readers with highest numbers of identical age estimates with the reference estimates had also the lowest bias from the reference estimate, but this was not always true (Wilcoxon's signed ranks test).

In the **Polish sample**, 64–91% of the fish were aged similarly to the mode, and only two readers aged at least 80% similarly to the mode. With half of the readers, their estimates were statistically significantly different from the mode. When the estimates were compared to one of the Polish readers with more experience from this population, 41–92% were identical to the determinations of this reader. Again, the two readers most experienced with this particular population, had the highest agreement with each other (92%), whereas others were in a lower level (41–76%). The results of all readers but one (76% agreement) were statistically different from the referred reader (Wilcoxon's signed ranks test).

The comparison did not give an answer to whether modal ages or the ages determined by the most experienced reader with the sample in question, are closest to the real ages of the fish. However, the readers most familiar with the sampled fish had high levels of agreement with each other, even though they could differ more from the other readers. This can express a better knowledge on the characters of a particular population or just higher agreement among the experienced readers, based on common experience in learning the interpretation.

Examination of otoliths together:
Despite the differences in the age determination results of the otolith exchange, the interpretation of rings was fairly unanimous when observing the otoliths projected on the wall together. The participants agreed fairly easily on the interpretation on annual rings, this for all population samples. However, there were differences in the interpretation of age from the rings. Some

variation was found in the definition of birthday, especially in autumn spawning herring that do not have a full year of life behind in the general birthday of fish, 1 January (see 4.1.1).

2) Otoliths from specimens from which both otoliths had been taken for the examination: one as whole and one as sliced and stained with neutral red

Most otoliths were examined as whole, but samples from several populations had been taken for sawing and slicing, and the thin slices had been stained with neutral red. In a sample from the North Sea, all rings were detectable in both whole and stained otoliths. Most samples that were examined were from the Baltic Sea. In slowly grown herring, all rings could not always be identified in the whole otolith, whereas they could be seen at the edge of the stained otolith cross sections.

A special example was the Gulf of Riga, where a modification of counting rings from whole otoliths is in use (G. Kornilovs): in addition to the number of rings, the width and structure of the visible growth zones are included in the determination.

An example: 40 Gulf of Riga herring otoliths collected in January 2005 had been sent to Finland for staining. The ages of these herring were determined also by the conventional method under the stereo microscope. Taking into account the present otolith formation peculiarities of the Gulf of Riga herring, it was often not possible to determine age by counting hyaline rings in the conventional way, but the age of the herring was determined considering that the transition zones have formed in a defenite year. At the workshop, the images of stained otolith slices were shown on the screen, and the age was determined and compared with the conventionally determined age. Due to time constraint, only 10 herring otolith slices were observed and in all cases but one the ages coincided. In one case, conventionally determined age was 4 whereas age from stained otolith slice was 5. It was not possible to conclude whether this difference was because this otolith had a false ring, which on the otolith slice was defined as a ring or because this otolith has unusually narrow growth zone of 2002 which was very good growth year and is pretty wide on otoliths of year classes 2000 and 2001. In general, it could be concluded that both methods could be successfully used for age determination of herring.

In the Finnish samples from the Bothnian Sea that situates in the northern Baltic Sea, it is fairly usual that the whole otoliths of herring older than six years have a translucent zone at the edge of the otolith. This zone can be wider than the distance between two previous detectable rings. When examining the other otolith of the same fish as a thin slice with the cross section stained, a number of densely located rings are found from this area near the edge. Some rings may be detectable only in the inner edge of the sagitta, around sulcus. In the slow grown herring, the stained otolith cross section was generally clearer than the image of the whole otolith.

4. Conclusions and recommendations

A day in the meeting was used in subgroup working to discuss practical matters in the preparation of otoliths and age determination of herring. The group was divided in two on ecological basis: the Atlantic group and the Baltic group. The reports and recommendations of the subgroups are below as sections 4.1 and 4.2.

4.1 AGE ESTIMATION OF OTOLITHS (SAGITTAE) FROM ATLANTIC HERRING STOCKS (Chair Willie McCurdy)

4.1.1 Otolith Preparation

a. Extraction

i. Broken otoliths

Try to remove each otolith in one piece. If both otoliths are broken and there are enough fish of the same size remaining in the sample, pick another fish. If no substitute fish is available, collect all the otolith pieces and place them in the storage tray or envelope.

ii. Crystalline otoliths

If both otoliths are crystalline and there are enough fish of same size remaining in the sample, pick another fish. Crystalline otoliths should only be assigned an age by experienced readers, where evidence of all the translucent zones is visible. If the pattern of translucent zones is not clearly visible, no age should be assigned to these otoliths.

iii. Cleaning

Clean the otoliths in warm fresh water, to remove the risk of salt crystal formation. Ideally deionised water should be used. Change the water regularly. Dry the otoliths on dark coloured tissue paper and place them in the storage container or envelope. The interpretation of the implementation of health and safety regulations varies by national legislation and may not permit the use of alcohol without stringent controls. Additionally there is a risk that alcohol may effect the clarity of the translucent zones if the otoliths are stored in alcohol.

iv. Drying.

Make sure that otoliths are dry before embedding them in resin or observing them in baby oil.

v. Otolith Storage

It is recommended that plastic storage trays with protective lids, or specialist 'otolith' paper envelopes are used to store otoliths. Commercially available plastic trays with suitable storage cavities and custom made trays are both acceptable for otolith transport and storage. The storage of otoliths in plastic bags is not recommended, as these are unsuited to long-term otolith conservation.

b. Observation media

1. Histokitt and Entellan

The otoliths are placed in numbered cavities on moulded black plastic cards. The resin is added, the position of the otoliths adjusted if necessary and the resin is left to cure. No cover slip is used with these resins. Entellan is less toxic than than Histokitt, but has a higher shrinkage rate. Histokitt is no longer available in many European countries. All work is carried out in a fume cupboard. An additional dissadvantage of Histokitt is that the resin surface attracts dust and a clearing liquid may have to be used (e.g. water or baby oil). Several different numbering sequences are used at different laboratories and readers should be aware of this when reading exchange otoliths.

2. Polyester resin and glass cover slip

The otoliths are placed cavities on moulded black plastic cards. Occasionally machine-drilled Perspex cards are also used. The otoliths are placed cavities on the plastic cards. The cavities are filled with catalysed clear casting (polyester) resin and the position of the otoliths is adjusted using a seeker needle if necessary. Any repositioning of the otoliths must be completed before the resin begins to cure. Additional catalysed resin is poured on top of the card, to form a uniform layer and a custom cover slip is placed on top of the resin. The resin is then left to cure.

3. Hamburg Method

Special glass slides are prepared, by fixing thin black plastic strips to a custom-sized glass slide. The spacing between the black strips is the same as the width of the resin strips. The otoliths are mounted in pairs, one otolith of each pair on a black background and one on the clear glass. The otoliths are carefully covered with catalysed polyester resin and a custom sized glass cover slip is added. The slides are expensive to make, but placing one otolith on clear glass provides the additional advantage of using transmitted light to determine the nature of the otolith edge. Up to 100 pairs of otoliths can be mounted on each slide. Some readers in exchanges prefer to use slides or cards with a maximum of 25 otoliths.

4. Immersion in baby oil

This method is inexpensive and quick. The otoliths can easily be orientated within the light path, making it relatively simple to find the optimum viewing angle. It is simple to reposition any otoltihs which are not the right way up. Additional care is required when transporting otoliths stored in oil.

4.1.2 Age estimation

c. Glossary

The glossary published in the proceedings of the 1st Otolith Research Symposium, Hilton Head, 1993, should be used.

Kalish J.M. *et al*. 1995. Glossary for Otolith Studies. In *Recent Developments in Fish Otolith Research*. (D. H. Secor, J. M. Dean, S. E. Campana., eds) pp. 723-729. Univ. South Carolina Press, Columbia, SC., USA

d. Magnification and Illumination

When making an initial estimate of the number of translucent growth zones in an otolith, the minimum necessary level of magnification and illumination should be used. The magnification and/or illumination can then be increased to resolve specific features within the pattern of translucent and opaque growth zones.

e. Typical problems

If the otoliths have been properly cleaned and are well prepared, there are few problems in reading otoliths from Atlantic herring stocks.

f. Dissagreement in the interpretation of translucent zones

Few dissagreements on the interpretation of annual translucent zones exist between experienced readers, including the position of the first translucent zone, split translucent zones and the resolution of the otolith edge. However otolith readers are much more confident when reading their own stocks. Differing interpretations of the relationship between assigned age and year class caused significant variation in the assigned age for some otoliths.

g. Validation of winter zones

There is no direct validation of otoliths from Atlantic herring stocks at this time.

h. Spring spawning stocks

Some readers were unaware that a birthday of 01 April had been retained for the Celtic Sea Spring spawning herring stock.

i. Autumn spawning stocks

It should be noted that in certain Autumn spawning stocks e.g. Irish Sea (VIIa), some ‘0’ and ‘1’ group herring can complete the annual translucent zone for the current year before 01 January. If the date of capture is before 01 January these translucent zones should not be counted when assigning ages. In some herring older than age group ‘8’, the translucent zone for the current year may not be clearly visible until after 01 January. If the date of capture is before 01 January these translucent zones should be counted when assigning ages.

j. Mixed stocks

The year class is the year of birth for both autumn and spring spawning herring. It is important to identify the spring spawning component(s) of mixed stock samples in order to prevent the spring-spawned herring being assigned to the wrong year class.

Where samples contain herring from stocks that spawn at different periods, (e.g. autumn and spring spawning herring), it is recommended that an additional data field (e.g. stock code or spawning period), should be used to enable the stocks to be disaggregated for stock assessment purposes, where this is required. Where readers are unfamiliar with one or more components of mixed stock samples, they should seek advice from readers who have experience in stock differentiation methods. Most spring spawning herring otoliths are characterised by a small L1 within a relatively broad otolith, while most autumn spawning herring are characterised by a relatively large L1. This information should be considered in conjunction with biological information such as length and maturity stage, when assigning stock to individual fish.

4.1.3 Quality assurance and quality control

k. Written procedures

Written procedures should be produced for all otolith preparation and age estimation methods where the age data are used for stock assessment purposes.

l. Training

Written procedures must be supported by an effective training programme (a TACADAR CA recommendation). Particular attention should be paid to the need to preserve accumulated experience at each institute and adequate provision should be made to maintain succession of experienced readers.

i. Equipment

Discussion microscopes (Microscopes with twin optics) can be used to enable two readers to interpret the same otolith simultaneously. Video cameras, monitors and projectors may also be used for training and to agree reference set ages. Computer software can be used to annotate reference sets and training otolith images, by storing information (e.g. translucent zone positions) in layers that can be hidden or revealed.

m. Number of readers

For Quality Control it is recommended that a proportion of all herring ages used for stock assessment purposes, should be estimated by more than one reader at each institute.

n. Re-reading of otoliths

At institutes where only one person estimates the ages of herring otoliths, a proportion of the otoliths should be re-read to enable precision checks to be made.

o. Inter-calibration

It is recommended that regular otolith exchanges take place between institutes in order to detect precision drift in the age estimations.

p. Workshops

It is recommended that workshops take place when inter-calibration exercises indicate that may be a potential problem with the assigned ages.

4.2. AGE ESTIMATION OF OTOLITHS (SAGITTAE) FROM BALTIC SEA HERRING STOCKS. (Chair Georgs Kornilovs)

4.2.1 Coordination of Baltic Herring Age Determination

In the last decade the age determination of Baltic herring has been coordinated by Baltic Herring Age Reading Study Group (BHARSG) which was established by ICES in 1996. BHARSG worked by correspondence in 1997 (ICES 1997), then had two workshops in 1998 and 2000 (ICES 1998, 2000) and was dissolved by ICES in 2001. Since 1997 several exchanges of otolith samples have been performed and they are continued also after the dissolution of the Study Group. Since 2004 the exchange of herring otolith samples is supported by Baltic Sea Regional Project (BSRP) which has also financed the participation of the Russian expert in the Herring Age Reading Workshop in Seili, Finland. More detailed description of otolith preparation and storage and age estimation peculiarities in the Baltic Sea countries may be found in the Report of the Baltic Herring Age Reading Study Group (ICES 1998).

4.2.2 Otolith Preparation

i.Otoliths from herring samples

Both otoliths (sagittae) are removed from fresh or defrosted Baltic herring samples. The samples could be random including 50 to 100 fish and then otoliths from all fishes are taken or the samples could be length stratified when a fish sample is grouped in 0.5 cm classes and a definite number of otoliths are taken from each length class.

ii.Crystalline otoliths

The crystalline otoliths are usually stored together with other otoliths because they are usually not recognized during the removement from the fishes. It was decided at the BHARSG meeting that crystalline otoliths should be recorded and then discarded from the sample. Partly crystallised otoliths which are readable should be recorded and the age should be read.

iii.Otolith Storage

No special cleaning of removed otoliths is performed but they are relieved from any tissues and then put into the means of storage. In number of countries the otoliths are stored in paper envelopes, plastic bags or small plastic bottles, while others put the otoliths into holes of plastic plates in which otoliths can be covered with Canada balsam, eukitt or boat lacquer.

One of the otoliths can also be sawn to an 0.4 mm thick slice that is stained with neutral red (a modification from Richter & McDermott 1990). A number of slices, embedded in polyester resin, are sawn at a time. One dried polyester slice includes c. 10 slices of otolith cross sections.

4.2.3 Age estimation

i Glossary

During the meeting of BHARSG in 1998 a standardized terminology was accepted to be used for consistency among Baltic herring otolith age readers (ICES 1998).

ii Magnification and Illumination

All laboratories involved in age determination of Baltic herring examine the otoliths under a stereo (binocular) microscope in reflected light against a black background. The “free” otoliths are immersed in ethanol or in water while the otoliths which are placed in plastic plates and covered with Canada balsam or eukitt do not need additional preparation. The magnification used mainly depends on the size of otoliths and age of the fish.

The neutral red stained slices are examined on an object class, under a research microscope in transmitted light. Magnification varies from 40 to 400 times, depending on the difficulty of the otolith.

iii Typical problems

The typical problems in age determination have been described in the report of BHARSG (ICES 1998). Since the growth of Baltic herring and respective formation of otoliths in the Baltic Sea could be significantly influenced by the hydro-meteorological conditions it was recommended to have regular monthly sampling and collection of Baltic herring otoliths.

iv Dissagreement in in the interpretation of translucent zones

Dissagreements on the interpretation of annual translucent zones could exist between experienced readers, including the position of the first translucent zone and the resolution of the otolith edge. However otolith readers are much more confident when reading their own stocks (see Working Document by G.Kornilovs). When interpreting stained otolith slices, the familiarity of the stock is not as important as with whole otoliths.

v Spring spawning stocks

In the Baltic Sea the spring spawning herring is the dominating herring race. The spawning could start in the south western part of the Baltic Sea as early as in the end of February and could end in July in the north-eastern part of the Baltic Sea. Based on the internationally accepted standard they are assumed to have a birth date of January 1. Thus the age of the spring spawning herring shall be always equal with the number of hyaline rings and in the cases when after the January 1 the opaque zone of the previous year is not followed by a hyaline ring it should be included in the total number.

vi Autumn spawning stocks

At present the abundance of autumn spawning herring in the Baltic Sea is on a low level but this race was quite abundant till 1960s. Like the spring spawning herring it has a birth date of January 1 and it is related to the year of spawning. Since in autumn spawning herring otoliths the hyaline ring is not formed during the first winter, the age of the autumn spawning herring will equal the number of hyaline rings plus 1.

vii Mixed stocks

Several populations of Baltic herring are distinguished in the Baltic Sea differing by spawning place and time, migration pattern, growth rate and mean size at age. During the feeding season (summer-autumn) different populations are mixing thus causing additional problems in age determination. Otoliths of some fast growing and early spawning herring populations have large and wide first summer zone (L1) while other later spawning populations have relatively small first summer zone. This could lead to wrong assignation of the first hyaline ring.

4.2.4 Quality assurance and quality control

At present the quality assurance and control is provided at the national level and is not internationally postulated. The analysis of age determination exercises performed by BHARSG and the age determination results from otolith sample exchanges gives necessary indications where additional training or cooperation would be desirable. Since 1997 regular otolith exchanges (in total 6) took place between institutes in order to detect the changes in agreement in the age estimations of the national experts. It was recommended by BHARSG to have regular workshops at least once in three years, however, the last workshop was held in 2000.