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**Agri-Food and Biosciences Institute
Standard Operating Procedure**

SOP Code:	FAEB (Branch)	MARFISH (Unit/Group)	013 (SOP No.)	V8 (Version)
Location (e.g. Newforge Lane):	Newforge Lane			
Author:				
Title:	Age determination of Irish Sea herring			
Purpose: (please specify: analyse / measure / test / operate a method / equipment etc)	This procedure details the operations to be carried out in order to ensure that the following is conducted in a consistent manner: Age determination of Irish Sea herring			
Date of creation /amendment:	30/06/2014			

It is the project leader's responsibility to ensure that the appropriate SOP is specified for scientific work and that the SOP and training are provided to staff conducting the work. It is the responsibility of the operator to follow the method, to record which SOP is used and any deviation from the written SOP.

Procedure

Guidance:

- 12 *Standard operating procedures may be in numbered point format, with or without subheadings, or in a different format as appropriate to the work.*
- 13 *Any other documents referred to must be clearly cross-referenced.*
- 14 *If it is necessary to amend the SOP, a new version must be created and copied to all who use it. Old versions must be withdrawn and archived and dates of amendments recorded.*

Signed:	(author)	(date)
	(laboratory manager)	(date)
	(unit manager or project leader)	(date)

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1. **Scope**

This procedure uses the pattern of growth zones found in sagittal otoliths to determine the ages of autumn spawning herring from the Irish Sea Mourne and Isle of Man stocks.

The sagittae are the largest of the three pairs of otoliths found in herring, and the growth zones are commonly referred to as summer and winter rings.

2. **Field of application**

MARFISH010 is used to produce pairs of herring otoliths black plastic microscope slides.

The present procedure uses these slide mounted herring otoliths to determine herring ages.

Age data are used by the ICES Herring Working Group for the production of Irish Sea herring stock assessments.

3. **References**

MARFISH002: Sampling at sea aboard RV *Corystes*: pelagic fish.

MARFISH010: The production of slide mounted pelagic fish otoliths, (sagittae) for fish ageing.

MARFISHRA04: Marine otolith processing for age reading

MARFISHRA05: Otolith and shell age reading

MARFISHRA08: Using a microscope at a work bench

4. **Principle**

The age determination of fish by means of the study of otolith growth zones is simply a process of pattern recognition.

The number of valid winter growth rings is counted in order to determine the year class of each herring (the year in which an individual fish was spawned).

Winter rings contain a higher proportion of the protein "otolin" than summer rings. This gives the winter rings a bluish-grey appearance when viewed under incident light. These winter rings are more correctly described as hyaline rings. Summer otolith growth is more correctly described as opaque growth.

Fish age determination should be supported at intervals by validation assessments using material of known age from tagged or marked fish.

The accuracy of the outputs from this procedure should be verified by periodical exchange of samples with at least two other institutes, which have quality assurance schemes in force, and which carry out similar research.

5. **Reagents**

None.

6. **Equipment**

A low to medium power optical microscope with a zoom lens system.

Lightweight disposable gloves.

A small glass polishing cloth.

7. **Sampling**

Only otoliths which have been mounted correctly on black plastic slides as set out in procedure MARFISH010 may be used for age determination.

Not all otoliths which have been correctly mounted should be aged. Otoliths which may not be used for ageing include;

- (a) otoliths which display signs of calcium re-absorption,
- (b) otoliths with irregular growth patterns,
- (c) pairs of otoliths which have significantly different ring counts,
- (d) sagittae which are upside down, i.e. the face bisected by the sulcus acusticus is uppermost.
- (e) and damaged otoliths.

8. **Operational procedure**

8.1.1 Each microscope slide is placed on the viewing platform (stage), of a low power optical microscope which has been properly set up for the vision of the user. The magnification is set between X6 and X12, depending on the size of the otolith, and the clarity of the individual growth zones.

8.1.2 The number of winter rings visible on one axis of the otolith is counted using incident light.

8.1.3 The number of winter rings visible on a second axis of the otolith is counted using incident light.

8.1.4 The two axes used for winter ring counts may either be
From the nucleus to the rostrum,
or,
from the nucleus to the post-rostrum,
or,
from the nucleus to the anti-rostrum,
or,
from the nucleus to the para-rostrum.

8.1.5 Repeat 8.1.2. and 8.1.3. until either agreement or a difference of one is reached. Where satisfactory agreement cannot be reached, the otolith should be rejected as unreadable by means of optical microscopy alone.

8.2.1 The age value assigned to each fish is the "ring count". When the correct "ring count" is subtracted from the year in which herring was killed (the date of capture), the result will normally be the year the herring was spawned, (it's year class). Exceptions include late autumn spawned herring, where the year class may be calculated, by subtracting the value of the "ring count plus one", from the date of capture.

8.2.2 The correct "ring count" is determined by assigning all fish a birthday on 31 December at 24:00 hrs and by counting the number of valid winter rings.

8.2.3 The assignment of "ring counts" to herring otoliths may be facilitated by understanding the manner in which individual growth zones are laid down on the otoliths.

8.2.4 Such understanding may only be gained through a thorough study of the otoliths from each stock of each species of fish. It is necessary to study at least 5,000 otoliths from a herring stock in order to attain competence for that stock.

8.3.1 The following guide-lines will assist inexperienced otolith readers to age herring from the Irish Sea mourne and manx stocks.

8.3.2 Fish caught early in the year (January - May).

If the fish was very young (usually <1 year), and had summer zone growth on the edge of the otolith,

or,
if the fish was young (usually <2 years), and had winter zone growth on the edge of the otolith, then, the "ring count" is the total number of winter rings.

If the fish was older (usually fish of <5 years), and appeared to have summer ring growth on the edge of the otolith, then the "ring count" is the total number of winter rings, including the final and possibly incomplete winter ring on the edge.

In very old fish (<10 years), the last summer ring which follows this, may not be present until early spring, and may not be completed until early summer.

8.3.3 Fish caught later in the year (June - December).

If the fish was very young (usually <1 year) and had summer ring growth on the edge of the otolith, then the "ring count" is the number of complete winter rings minus one, (discount the premature winter ring which relates to the forthcoming winter)

If the fish was young (usually <2 years), and had "ring count" is the number of complete winter rings, last incomplete winter ring on the edge).

If the fish was older (usually fish of >5 years), and had summer ring growth on the edge of the otolith, then the "ring count" is the total number of winter rings.

In very old fish (<10 years), the last winter ring and the summer ring which follows this may be very narrow and difficult to observe.

8.3.4 In older fish some of the more recently deposited winter rings may show signs of splitting. Split rings are more visible on the rostrum, and care must be taken not to count each split rings as two separate winter rings.

9. Expression of results

The results are expressed as the "ring count" for each pair of herring otoliths, and this value is recorded on the relevant computer database.

10. Quality assurance

The outputs of this procedure (herring ages) may only be used for the purposes of fish stock assessment if they have been generated by, or have been verified by a competent otolith reader.

For any individual herring stock, a competent reader is defined as a person who has read at least 5,000 herring otoliths from that stock.

For each herring stock that is studied by particular laboratory, it is recommended that that laboratory should send at least three samples of 50 otoliths from that stock, to at least two other laboratories who also work on the same stock.

The receiving laboratory will randomly select the otoliths to be exchanged, from the lists of herring samples which have been collected by the transmitting laboratory.

The efficiency of the otolith exchange process may be considerably improved by the exchange of digitised images on magneto-optical discs, CD roms, or direct transfer by modem, where there is system compatibility between the institutes.

Regular otolith or digitised otolith image exchanges should also take place with other readers under the framework of the ICES Herring Assessment Working Group.

These additional otolith exchanges will help to maintain an acceptable rate of agreement between all the laboratories which produce age/length keys for the ICES Herring Assessment Working Group.

11. **Reporting of results**

The results are expressed as the "ring count" for each pair of herring otoliths, and this value is recorded on the relevant computer database.

12. **Safety**

The otolith sections mounted on the black plastic microscope slides must not be handled until the resin is completely cured.

Light weight disposable gloves must be worn by persons handling slides which contain cured resin.

The low powered optical microscope must be set up correctly in accordance with the manufacturer instructions for the operator, each time there is a change of operator.

Avoid long uninterrupted periods of microscope work by rotating tasks and taking frequent rest breaks. Every 15 minutes, close your eyes or focus on something distant. Care should be taken to ensure that light intensity is not excessive when observing specimens.

Use a designated microscope chair which provides correct ergonomic support. An adjustable footrest should be used to support feet. A break away from the microscope should be taken every 30-60 mins: get up to stretch and move.

Spread the work out doing a few hours daily over a number of days than trying to condense it all into a short period.