

AFBI adheres to ISO 9001:2008; an internationally accepted quality of standard. All activities are regularly monitored by an independent Quality Assurance Unit.

This document is an uncontrolled copy of an AFBI standard operation procedure (SOP) and should be treated as a draft. AFBI has a dedicated laboratory manager whose responsibility it is to update SOPs on an internal Quality Management System (QMS) which may not be reflected in this document.

This document is for reference purposes only.

Agri-Food and Biosciences Institute

Standard Operating Procedure

SOP Code:	Fisheries & Aquatic Ecosystems Branch	MARFISH (Unit/Group)	011 (SOP No.)	V7 (Version)
Location:	Newforge Lane, Belfast, BT9 5PX			
Author:				
Title:	Age determination of Irish Sea demersal fish			
Purpose:	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 demersal fish			
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 the SOP that is used and any deviation from the written SOP.

Guidance:

1. *Standard operating procedures may be in numbered point format, with or without subheadings, or in a different format as appropriate to the work.*
2. *Any other documents referred to must be clearly cross-referenced.*
3. *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 Old versions must be withdrawn and archived and dates of amendments recorded.*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)

CONTENTS

Section	Topic
1	Scope
2	Field of Application
3	References
4	Principle
5	Reagents
6	Equipment
7	Sampling
8	Operational Procedures
9	Expression of Results
10	Quality Assurance
11	Reporting of Results
12	Safety

1. **Scope**

This procedure uses the pattern of growth zones found in sagittal otoliths to determine the age of demersal fish from Irish Sea (ICES area VIIa).

The principal Irish Sea demersal fish stocks studied at the FAEB Marine Fisheries laboratory are cod, haddock, and whiting.

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

2. **Field of application**

The sagittal thin sections mounted on glass microscope slides obtained through MARFISH016 are used to determine age of demersal fish.

3. **References**

MARFISH003: Sampling at sea aboard RV Corystes: demersal fish.

MARFISH017: Sampling the N. Ireland landings of demersal fish.

MARFISH037: Collecting and Processing Enhanced Discard Samples.

MARFISH016: Otolin System for the Embedding, Sectioning and Slide Mounting of Demersal Fish Saggitae (otoliths).

MARFISHRA04: Marine otolith processing for age reading.

MARFISHRA05: Otolith and shell age reading.

MARFISHRA08: Using a microscope at a work bench.

4. **Principle**

4.1 Demersal fish landings by the N. Ireland fishing fleet into N. Ireland fishing ports, are recorded in order to monitor the application of demersal fish TACS.

4.2 Demersal fish samples are sampled at sea (procedure MARFISH003), and at N. Ireland fishing ports (procedures MARFISH017 and 037), in order to collect fish otoliths and biological information.

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

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

4.5 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.

- 4.6 These demersal fish age determinations must be supported at intervals by validation assessments using material of known age from tagged or marked fish.
- 4.7 The accuracy of the outputs from this procedure must be verified by an annual exchange of samples with at least two other institutes, which have quality assurance schemes in force, and which carry out similar research.
- 4.8 FAEB Marine Fisheries otolith readers will take part in all Cod, haddock, hake, and whiting otolith exchanges organised by ICES Working Groups

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 dorso-ventral otolith thin sections which have been mounted correctly on glass slides in accordance with procedure MARFISH016, 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 at this laboratory include:

- (a) otoliths which display signs of calcium re-absorption,
- (b) otoliths with interrupted growth patterns,
- (c) otoliths which have not been sectioned through the centre of the nucleus,
- (d) damaged otoliths.
- (e) otolith sections that are thinner than 0.28 mm,
- (f) otolith sections that are thicker than 0.38 mm,

(g) and otolith sections with insufficient black pigment in the embedding media.

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 cross section are counted using incident light.
- 8.1.3 The number of winter rings visible on a second axis of the otolith cross section is counted using transmitted light.
- 8.1.4 The two dorso-ventral axes used for winter ring counts may be located in either,
the nucleus to the dorsal anterior quadrant,
or,
the nucleus to the dorsal posterior quadrant,
or,
the nucleus to the ventral anterior quadrant,
or,
the nucleus to the ventral posterior quadrant.
(the lower face of the saggitae is the face which is bisected by the sulcus acusticus, figs, 1 & 2.)
- 8.1.5 Turn the slide over and repeat 8.1.2. and 8.1.3. Repeat 8.1.5. until agreement is reached. Where agreement cannot be reached, the otolith should be rejected as un-ageable by optical microscopy alone.
- 8.1.6 The growth rings on Irish Sea whiting otoliths are often only visible on the lower dorsal and the lower. The age value assigned to each fish is the "age group". When the correct "age group" is subtracted from the year in which the fish was killed (the date of capture), the result will be the year the fish was spawned, (it's year class).

- 8.2.1 The correct "age group" is determined by assigning all fish a birthday on 31 December at 24:00 hrs. and by counting the number of valid winter rings.
- 8.2.2 The assignment of "age groups" to demersal fish otoliths may be facilitated by understanding the manner in which individual growth zones are laid down on the otoliths.
- 8.2.3 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 stock of a demersal fish species in order to attain competence for that stock.
- 8.3.1 The following guide-lines will assist inexperienced otolith readers to age demersal fish from Irish Sea (ICES area VIIa) fish 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 "age group" 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 "age group" 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 maybe very narrow and difficult to observe. The winter 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 "age group" 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 winter ring growth on the edge of the otolith, then the "age group" is the number of complete winter rings, (discount the 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 "age group" 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 Some very young whiting (<1 year), can lay down a complete hyaline ring, and deposit additional opaque growth on the otolith, before the end of December. This premature winter (hyaline) ring must not be counted in fish caught before 31 December. The additional opaque growth is easily identified as such, as this is rarely larger than 20% of the previous complete summer's growth.

8.3.5 In some otoliths, the winter rings may show signs of splitting, and in whiting these split winter rings may be broader than the summer rings. Care must be taken not to count each split ring as two separate winter rings.

8.3.6 In some whiting and haddock otoliths, the first winter ring is composed of two separate narrow and such winter is two winters. More rarely the same phenomenon is also observed in the second winter ring.

8.3.7 In Irish Sea cod the first winter ring is often relatively faint. In cod this is a common feature of winter rings which are laid down prior to the age at first maturity. Care must be taken not to confuse the first wintering with the very narrow post nucleus settlement ring which precedes it.

8.3.8 In Irish Sea hake otoliths, the summer rings contain many diffuse hyaline rings. This gives rise to the false impression that these otolith sections have broad winter rings and narrow summer rings. The winter rings in these hake, although often difficult for an inexperienced reader to discern, are very fine, distinct and relatively brighter than the other more diffuse hyaline rings.

9. **Expression of results**

The results are expressed as the "age group" for each demersal fish otolith, and this value is recorded on the FAEB marine fisheries database.

10. **Quality assurance**

- 10.1 The outputs of this procedure (demersal fish 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.
- 10.2 For any individual demersal fish stock, a competent reader is defined as a person who has read at least 5,000 otoliths from that stock.
- 10.3 For each demersal fish 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.
- 10.4 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.
- 10.5 The efficiency of the otolith exchange process may be considerably improved by the exchange of digitised images on magneto-optical discs, CD ROM's, or direct transfer by modem, where there is system compatibility between the institutes.
- 10.6 Regular otolith or digitised otolith image exchanges, must take place each year. These otolith exchanges will help to maintain an acceptable rate of agreement between all the laboratories which produce age/length keys for the ICES Northern Shelf Working Group.

11 **Reporting of results**

All results must be directly entered on to the FAEB marine fisheries database.

12. **Safety**

The otolith sections mounted on the glass 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 manufacturers 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.