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

SOP Code:	FAEB (Branch)	MARFISH (Unit/Group)	016 (SOP No.)	V2 (Version)
Location (e.g. Newforge Lane):	Newforge Lane			
Author:				
Title:	Otolin System for the Embedding Sectioning and Slide Mounting of Demersal Fish Sagittae (Otoliths).			
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: Otolin System for the Embedding Sectioning and Slide Mounting of Demersal Fish Sagittae (Otoliths).			
Date of creation /amendment:	01/03/2014			

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:

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 Old versions must be withdrawn and archived and dates of amendments recorded.*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

THE FISHERIES AND AQUATIC ECOSYSTEMS BRANCH (FAEB) OTOLIN SYSTEM.

Otoliths samples are collected at fish markets, from self-sampling and during RV *Corystes* research cruises. By 1998, the number of demersal otoliths processed annually had far exceeded the sectioning capacity of the existing Isomet slow speed saw.

1 SCOPE OF THE OTOLIN PROCEDURE

- 1.1 Otoliths from demersal fish samples are embedded within polyester resin blocks.
- 1.2 Thin-sections containing otolith centres are cut from the resin blocks. Otolith thin-sections are mounted on microscope slides.

2 FIELD OF APPLICATION

Otoliths and biological data are collected from samples of demersal fish.

- 2.1 Part one of this Procedure describes the method for embedding demersal fish otoliths in black resin blocks.
- 2.2 Part two of this Procedure describes the method for cutting thin sections through the transverse centres of otoliths embedded in resin blocks.
- 2.3 Part three of this Procedure describes the method used to mount the otolith thin sections on glass microscope slides.
- 2.4 The slide-mounted otolith sections produced by the OTOLIN process are used for the determination of fish ages as described in MARFISH011.

3 PRINCIPLE

- 3.1 Demersal fish landings by the N Ireland fishing fleet are sampled at N. Ireland ports, and demersal fish catches are sampled at sea on board the Marine Research Vessel *Corystes*.
- 3.2 Individual fish from demersal fish samples are assigned a unique identification number based on sample type, species code, year date, sample number, and fish number within the sample. Biological data for each fish are logged on to the appropriate marine fisheries database.

- 3.3 Pairs of otoliths (sagittae) are dissected from individual fish demersal fish samples, and stored in individual compartments in plastic boxes (Repli dishes), prior to embedding in resin blocks in accordance with this Procedure.
- 3.4 The resin blocks are thin sectioned and mounted between glass microscope slides and cover-slips.
- 3.5 The mounted otolith thin sections are then used to determine fish ages for individual fish records in accordance to MARFISH011.

4 REAGENTS

- MarPlas black polyester resin kit for otolith embedding.
- MarPlas Tack-free black polyester resin kit for otolith embedding.
- MarPlas clear casting polyester resin for otolith embedding.
- "Poly-Pol" PS6 polyester laminating resin (lamineerhars) or "Lay-up" resin.
- "Poly-Pol" PS28 polyester gel coat resin (gielhars) or "Casting" resin.
- "Poly-Pol" PS230 polyester Flex resin (flexible menghars).
- "Ryland's" Black colour paste for polyester resin or "Poly-Pol" pigment paste 01.
- Methyl ethyl ketone peroxide (MEKP resin catalyst, a.k.a. hardener).
- Commercial "spray on" mould release agent or poly vinyl adhesive (PVA).
- Powdered talc (French chalk).

5 EQUIPMENT

5.1 OTOLIN OTOLITH PROCESSING LINE EQUIPMENT.

5.1.1 Otolith Embedding Equipment:

- OTO 910 Levelling Unit for casting resin in the moulds
- OTO 919 Mini Fume Hood for OTO 910 Levelling Unit
- OTO 914 *Double Moulds (a minimum of 4 is recommended)
- OTO 920 Mounting Station - comprising,
- OTO 918 Mini Fume hood with extraction adapter

5.1.2 Sectioning Equipment:

- OTO LABCUT 230F floor standing abrasive cutting machine
- Otolith sectioning jig
- Diamond wafering blade
- Slide Preparation Equipment
- OTO 926 Slide mounting kit
- OTO 911 Levelling station for slide preparation

- OTO919 Mini Fume hood for slide preparation
- 5.1.3 Section Mounting Equipment and Materials:
- Glass microscope slides (76mm X 50mm 1.0mm).
 - Glass microscope cover-slips (76mm X 50mm 0.5mm)
- 5.1.4 Quality Control Equipment:
- Measuring Calipers - minimum resolution 0.01 mm.
- 5.1.5 Each OTOLIN aluminium double mould is engraved with an identification letter and each half of the mould base is engraved with an identification number.
- 5.1.6 A suitable Laboratory fume cupboard may be used in place of the Fume Hoods.

6 Part 1: EMBEDDING THE OTOLITHS IN RESIN BLOCKS.

6.1 OPERATIONAL PROCEDURE.

Polyester Resin Mixing Table for the Bottom (first) Resin Layer:

Weight (gr.)	5	10	20	30	40	50	60	70	80	90	100	120	140	160	180	200
Lay-Up Resin	4.5	9	18	27	36	45	54	63	72	81	90	108	126	144	162	180
Flex Resin (5%)	0.25	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0
Black Resin Pigment (5%)	0.25	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0
Hardner (EMKP) (1%)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.4	1.6	1.8	2.0

Otolith log book example.

Mould Number			
Date		Operator	
Left Section		Right Section	
CUTTING LINE	OTOLITHS EMBEDDED	CUTTING LINE	OTOLITHS EMBEDDED
Row 1		Row 1	
Row 2		Row 2	
Row 3		Row 3	
Row 4		Row 4	
Row 5		Row 5	
Row 6		Row 6	

Polyester Resin Mixing Table for the Top (second) Resin Layer:

Weight (gr.)	5	10	20	30	40	50	60	70	80	90	100	120	140	160	180	200
Casting Resin	4.5	9	18	27	36	45	54	63	72	81	90	108	126	144	162	180
Flex Resin (5%)	0.25	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0
Black Resin Pigment (5%)	0.25	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0
Hardner (EMKP) (0.6%)	0.03	0.06	0.12	0.18	0.24	0.30	0.36	0.42	0.48	0.54	0.60	0.72	0.84	0.96	1.08	1.20

6.2 EXPRESSION OF RESULTS

Each otolith is assigned a unique identification code and each resin block section is clearly labelled with the identity of the otoliths that are embedded within the block.

The identification codes engraved on the mould bases provide a moulded mirror image of these marks on the underside of each section of resin block.

6.3 QUALITY ASSURANCE

Check the number of otoliths in each sample against the appropriate sampling record book before beginning work.

Follow the procedure.

Check that the otoliths have not been disturbed, and record the sample number, and the fish record numbers for the otoliths in each cavity of the mould on a card, before adding the top layer of catalysed resin. Tape the card to the mould, until the resin blocks have completely set and have been engraved with the identity of the otoliths embedded within the block.

Inexperienced personnel should only attempt using one half of a mould at a time, until they gain the necessary speed to use a complete mould at one time.

6.4 SAFETY.

All COSHH regulations must be adhered to when carrying out this procedure. These are attached as appendix 1 of this procedure

ALL WORK EXCEPT THE ENGRAVING OF CURED RESIN BLOCKS MUST TO BE CARRIED OUT INSIDE THE FUME HOODS OR INSIDE AN APPROVED FUME CUPBOARD.

Disposable plastic gloves must be worn when spraying mould release and disposable plastic gloves suitable for use with styrene must be worn when mixing and pouring resin.

A non toxic particle mask, ("3M" type 8500 or better) must be worn when engraving the resin blocks.

See risk assessment documents:

MARFISHRA04: Marine otolith processing for age reading

MARFISHRA05: Otolith and shell age reading

7 PART 2: CUTTING OTOLITH SECTIONS FROM RESIN BLOCKS.

The OTOLIN saw (a Labcut 230F high-speed cutting machine), is powered by a 3-phase electrical motor. ONLY COMPETENT TRAINED PERSONNEL MAY USE THE OTOLIN CUTTING MACHINE.

IT IS ILLEGAL FOR PERSONS UNDER THE AGE OF 18 TO USE THE OTOLIN CUTTING MACHINE UNLESS THEY ARE SUPERVISED BY A COMPETENT TRAINED OPERATOR AT ALL TIMES.

7.1 SETTING UP THE LABCUT 230F HIGH-SPEED CUTTING MACHINE

Demersal Switch the power Isolation switch at the rear of the Lab-Cut machine to the "On" position.

Raise the safety hood and make sure the cutting fluid nozzles are both pointing along the cutting line.

Switch on the cutting fluid pump, lower the cutting head to the cutting position and check that the cutting fluid is spraying against the both sides of the blade.

- The cutting fluid must spray against the both sides of the blade during cutting to prevent blade damage.

Switch off the cutting fluid pump and raise the cutting head to top position.

7.2 CUTTING THE OTOLITH SECTIONS FROM THE RESIN BLOCKS.

Place the resin block in the machine, with the marked sections of equal width next to the cutting slot and tighten the screw that holds the block in place.

Lower the cutting machine hood.

First switch on the cutting fluid pump.

The cutting fluid pump must be switched on before the cutting blade motor, to ensure that the cutting fluid is running freely before the blade starts to cut.

Now switch on the cutting blade motor.

Use the operating lever to make the first cut. This cut will expose the centre line of the first row of otoliths.

The signal light will come on when the cut is finished after approximately 15 seconds. You will also hear the change in the sound made by the blade.

Now operate the lever to move the cutting blade to the top position.

Switch off the blade motor and the fluid pump.

Wait a few seconds and then open the door. There is a safety interlock on the safety hood - you cannot open this when the cutting blade is switched on.

Remove the first waste piece and place this on the work bench. This piece contains the first row of otolith halves.

Place the aluminium measuring block where the waste piece was positioned, loosen the clamping screw which holds the resin block and slide the resin block until it presses firmly against the aluminium measuring block. The cutting line indicator should now be pointing exactly at one of cutting lines engraved on the resin block.

Tighten the screw that holds the resin block in place and lower the safety hood.

Switch on the cutting fluid pump.

Now switch on the cutting blade motor.

Use the operating lever to make the next cut. This will make the first strip of otolith sections.

The signal light will come on when the cut is finished after approximately 15 seconds. You will also hear the change in the sound made by the blade.

DO NOT OPERATE THE LEVER TO MOVE THE CUTTING BLADE AWAY FROM THE BLOCK. Switch off the fluid pump and the cutting blade motor.

When the machine has stopped and the safety interlock has switched off, raise the safety hood and operate the lever to lift the cutting blade away from the resin block. The blade is now in the top position and the section is usually attached to the edge of the blade by the moisture in the cutting fluid.

Gently remove the section and use a measuring gauge to check that the section thickness is within the required tolerance range.

Remove the aluminium measuring block from the machine

Repeat previous steps until all the sections have been cut from the block.

Repeat the entire sequence for all the resin blocks.

Switch the power Isolation switch at the rear of the Lab-Cut machine to the "Off" position.

7.3 EXPRESSION OF RESULTS

The cut sections and waste pieces may be set together in sequence on the bench top for mounting at a later time.

7.4 QUALITY ASSURANCE

Check that the saw is set up in accordance with this procedure.

Follow the procedure.

Use Vernier calipers to check the section thickness at the beginning and end of each cutting session – this should be 0.4 mm ± 0.05 mm.

7.5 SAFETY

Only trained personnel may use the cutting machine. Persons under the age of 18 must be supervised at all times.

Ensure that all equipment is properly maintained.

See risk assessment documents:

MARFISHRA04: Marine otolith processing for age reading

MARFISHRA05: Otolith and shell age reading

8 OTOLIN PART 3: MOUNTING OTOLITH THIN-SECTIONS ON GLASS SLIDES

8.1 OPERATIONAL PROCEDURE

Using the Powerline® Handy Engraver, engrave a glass microscope slide (76mm x 50mm) with the identification numbers of the otolith sections to be mounted on it. (This is precisely the same number found on the resin block. see section 7. A non toxic particle mask, ("3M" type 8500 or better) must be worn when using the Powerline® engraver. Rub black wax crayon into the engraving to make the legend more legible or write otolith information using a permanent marker on the coverslip. Set the resin block section(s) on the glass microscope slide. A maximum of four block sections can be mounted on one slide. Do not mix otoliths from different species on the same slide. All operations involving the mixing, pouring and setting of resin must be carried out inside an approved fume cupboard. Pour 25g of clear casting resin into a 250ml plastic beaker and add 0.2g M.E.K.P. catalyst. Stir gently to avoid introducing bubbles into the resin.

Using a 7ml "Liquipette" plastic disposable pipette, drop the clear catalysed resin onto the glass slide ensuring that all sectioned otoliths, and the spaces

between the resin strips are covered, to form a complete layer of catalysed resin on top of the slide.

Allow approximately 20 minutes (the time varies with the age and brand of resin used) for the resin to thicken. Gently place a glass microscope cover slip (76mm X 50mm X 0.5mm) on top of the resin and set the completed slide on a mounting pillar on the slide preparation mounting station. Leave in the fume hood or cupboard overnight at room temperature or until the resin has set.

The mounting pillars on the slide preparation mounting station allow excess resin to drip clear of the finished slides. Any resin drips can be removed from the floor of the mounting station once the resin has hardened.

8.2 EXPRESSION OF RESULTS

Each glass microscope slide will be clearly labelled with the unique identification numbers which refer to the otoliths mounted on it.

8.3 QUALITY ASSURANCE

Follow the procedure

8.4 SAFETY

All operations involving the mixing, pouring and setting of resin must be carried out inside an approved fume cupboard or fume hood.

All COSHH regulations must be adhered to when carrying out this procedure. These are attached as appendix 1. of this procedure as specified by the product suppliers.

All mounting work must be carried out in a fume cupboard.

Disposable plastic gloves suitable for use with Styrene must be worn when mixing and pouring resin.

A non toxic particle mask, ("3M" type 8500 or better) must be worn when engraving the resin blocks and glass microscope slides.

See risk assessment documents:

MARFISHRA04: Marine otolith processing for age reading

MARFISHRA05: Otolith and shell age reading

9 **REPORTING OF RESULTS.**

The glass microscope slides produced by this process, are used for fish age determination (MARFISH011)

10 **REFERENCES.**

MARFISH011: Age determination of Irish Sea demersal fish

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

MARFISH006: Sampling at sea aboard RV Corystes: Nephrops

MARFISHRA04: Marine otolith processing for age reading

MARFISHRA05: Otolith and shell age reading