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This document is for reference purposes only.
Agri-Food and Biosciences Institute
Standard Operating Procedure

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<th>SOP Code:</th>
<th>FAEB (Branch)</th>
<th>MARFISH (Unit/Group)</th>
<th>014 (SOP No.)</th>
<th>V7 (Version)</th>
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**Location** (e.g. Newforge Lane): Newforge Lane

**Author:**

**Title:** Embedding fish otoliths in resin blocks

**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: Embedding fish otoliths in resin blocks

**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 Old versions must be withdrawn and archived and dates of amendments recorded.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:**

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1. **Scope**
The procedure covers the production of resin blocks containing demersal fish otoliths dissected from samples, which have been logged on Marine Fisheries database.

2. **Field of application**
Samples of demersal fish otoliths, and these are stored in "repli" dishes. This procedure uses otolith samples to produce otoliths embedded in black resin blocks. These resin blocks are used by procedure MARFISH015 to produce slide mounted otolith sections for demersal fish ageing.

3. **References**
MARFISH015: The production of slide mounted demersal fish otoliths, (sagittae thin sections) for fish ageing.
MARFISH011: Age determination of Irish Sea demersal fish.
MARFISHRA04: Marine otolith processing for age reading
MARFISHRA05: Otolith and shell age reading

4. **Principle**
Individual fish from demersal fish samples are uniquely numbered and biological data for individual fish are logged on to databases. Pairs of sagittae are dissected from individual fish, and stored in compartmentalized trays prior to embedding in resin blocks in accordance with this SOP. The resin blocks produced by this procedure are subsequently thin sectioned and mounted between glass microscope slides and cover-slips in accordance with MARFISH015. Mounted sagittae thin sections are then used to determine fish ages for individual fish, in accordance with MARFISH011, Age determination of Irish Sea demersal fish.

5. **Reagents**
Brand "X" pre-accelerated polyester resin.
Methyl ethyl ketone peroxide (resin catalyst).
"Ryland's" Black colour paste for polyester resin.
Commercial "spray on" mould release agent.
Powdered talc (french chalk).
Uncooked spaghetti pasta.

6. **Equipment**
Laboratory fume cupboard.
Aluminium moulds engraved with unique identification codes. Individual moulds are engraved with an identification letter and each cavity is engraved with an individual identification number.
"Powerline" handy engraver.
Glass microscope slides (76mm X 50mm 1.0mm).
Glass microscope cover-slips (76mm X 50mm 0.5mm)

7. **Sampling**
The samples which are used as the inputs for this procedure, are the "repli" dishes of demersal fish otoliths. "Repli" dishes are clear plastic boxes supplied with lids, which are sub-divided into 25 compartments.

8. **Operational procedure**
8.1 Each "repli" dish of otoliths must be allowed to dry for a minimum of five days at room temperature, or 48 hours at 28°C in a drying oven prior to commencement of this procedure.

8.2 The mould is prepared for use by spraying each cavity lightly with mould release agent. **All operations involving the mixing, pouring and setting of resin must be carried out inside an approved fume cupboard.** Use a disposable plastic beaker to mix the resin. Add 0.2g of M.E.K.P. catalyst for each 25g of resin and stir gently to avoid introducing bubbles into the resin, until well mixed. A 3mm deep layer of catalysed resin is poured into each cavity of one of the aluminium moulds, and allowed to gel at room temperature (16°C-20°C). This process takes 10-25 minutes. A short length (<20mm) of uncooked spaghetti pasta is then placed on top of the resin at the left hand side of each mould cavity.

8.3 A row of sagittae is then set on top of each layer of gelled resin so that the nucleus of each sagitta lies directly along the cutting line which marks the longitudinal centre of each mould cavity. Each sagitta is individually placed on top of the resin, so that the interruption of the sulcus acusticus which indicates the position of the nucleus lies directly along the cutting line. This ensures that each otolith will be thin sectioned through the nucleus when the resin blocks are sawn through their longitudinal axes. Cod and other large sagittae are mounted with the concave face uppermost (ie sulcus acusticus facing downwards) to avoid the entrapment of air bubbles underneath the sagittae.

8.4 As the row of sagittae in each cavity is completed, the unique identification numbers of the sagittae, and the reference number of the mould cavity which contains them are recorded in the otolith embedding logbook.

8.5 When all the cavities in the mould have been filled with otoliths, a further layer of catalysed resin is poured over each row of otoliths, and the resin left to complete polymerisation at room temperature.

8.6 When the resin blocks have completely cured each block has a label indicating the content.

9. **Expression of results**
Each resin block will be clearly labelled with its unique identification number.

The unique identification number for each block comprises the species code, the year of sampling, the sample number, and the fish numbers for the otoliths embedded in the block.

10. **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.

Inexperienced personnel should only attempt part moulds (two to six cavities) until they gain the necessary speed to use a complete mould at one time.

11. **Reporting of results**
The outputs (resin blocks) produced by this procedure, are the inputs (samples) required for procedure MARFISH016. The resin blocks are thin sectioned prior to mounting on glass microscope slides in accordance with MARFISH016.
12. **Safety**

All COSHH regulations must be adhered to when carrying out this procedure. **All work except the engraving of cured resin blocks, must be carried out in 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.