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A new method of preparing gadoid otoliths

by

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Abstract

Modification of an existing technique of producing thin cross-sections of otoliths for age determination is described. Details of equipment are provided and a comparison of age determinations from thin sections and broken otoliths is made. An intra-reader agreement of 80-90% for cod, haddock and pollock was found. The new technique has been adopted as the standard for preparation of otoliths for cod, haddock and pollock by Marine Fish Division personnel.

Résumé

On a modifié une technique pour faire des coupes transversales minces dans les otolithes en vue de déterminer l'âge des spécimens étudiés. On donne des détails sur l'équipement employé et l'on compare la détermination des âges d'aprés des coupes minces avec les résultats obtenus par l'étude de fragments d'otolithes. On a constaté que les mesures concordent entre elles à 80-90 % pour la morue, l'aiglefin et la goberge. Cette nouvelle technique est maintenant la méthode standard qu'on emploie pour la préparation des otolithes de morue, d'aiglefin et de goberge à la Division des poissons marins.

Introduction

A new technique of preparing otoliths from cod, haddock and pollock for age determination was used by Marine Fish Division in 1984. The technique provides a thin sawed cross-section of otolith specimens in lieu of the broken cross-section obtained by the traditional method. This report describes the sectioning technique as a modification of one developed by Bedford (1983), and compares it with the traditional breaking technique on the basis of time, costs, viewing quality, shelf-life, storage, and impact on age data.

Sectioning Machine

A Herbert surface grinder was adapted to accommodate a diamond-edged wheel specially engineered by Norton Canada Ltd.[1] to cut a conglomerate of polyester and aragonite (Fig. 1). An irrigation system of water jets and a splash-tray was installed on the cross-sliding table for cooling and lubrication of the blade. A vise was installed in the splash-tray as a holdfast for material being sectioned. A ½-H.P. electric motor was adapted to provide an adequate spindle speed for smooth cutting.

Operation of the device requires little instruction. A single lever is used to advance the work into the saw, and an indexing wheel is advanced to gauge the thickness of the cut.

Mold Construction

A single compartmented mold producing a 127 mm x 152 mm x 12 mm block has proven adequate to accommodate the largest of gadoid otoliths. Aluminum has been demonstrated as the best choice of construction material, as it is durable and sufficiently corrosion resistant. Acrylic molds were tested over a 4-month period, but were found to corrode due to an interaction with the resin. Cracks were noted in the sides and bases after normal use of these molds, and acrylic was subsequently rejected as a construction material.

The mold is assembled with machine screws which facilitates quick block removal of the resin block by detaching one or more sides. The base and sides of the mold are etched with five reference lines, as shown in Fig. 2, to imprint the blocks as cutting guides.

Setting Up the Mold

A clear casting polyester resin, pigmented black and catalyzed to gel within 30 minutes, is used as an embedding medium. Common paste wax is used as a releasing agent in the mold, and the first layer of resin is normally restricted to less than 1/4 the total mold volume so as to allow adequate space for large otoliths.

Correct sequencing of sections (to be later removed from the completed resin block) is accomplished by placing a piece of uncooked spaghetti diagonally left to right in the mold within the first resin layer. The

[1] Norton Canada Limited
Hamilton, Ontario, Canada

result, after sectioning, is the appearance of a dot at the bottom of each section, and its position relative to the right-hand side of the section identifies the correct sequence of the section.

After the resin has gelled to the tacky stage, mounting lines are scribed into the first resin layer by referring to the etched cutting lines on the sides of the mold. Otoliths are arranged with the colliculum (the constriction in the sulcus on the proximal side) adjacent to the mounting line. Otoliths may be oriented according to reader preference. These choices include: a) in pairs with the proximal sides facing and either the dorsal or ventral edges oriented upward; and b) as singles with the proximal side downward. It is not recommended to place the distal sides (concave) down, as entrapment of air bubbles in the resin will result in holes through the sections. Due to resin cost, otoliths are spaced very closely, and a filled mold accommodates an average of 75 pairs of otoliths. The final resin layer is poured when all lines are filled, the block is numbered, and all relevant information is recorded for future reference.

Sectioning and Mounting

Blocks are removed from the molds and inverted to reveal cutting lines. Squaring a block within the carriage vise ensures the correct alignment of cutting lines to the blade. Sections can be produced without breakage or shattering to a minimum of 0.5 mm although 1.0 mm is the standard thickness. The volume and quality of sections is largely a function of the operator's dexterity and experience.

Sections are correctly sequenced as they are produced, and are later grouped by sample numbers. Sections are thoroughly cleaned with a soft nylon brush and water to remove all traces of sawdust, and are allowed to dry.

Mounting templates are made from sheets of 2-mm clear acrylic, and are prelabelled using self-adhesive labels with sample identification. The sections are cemented to the templates with transparent spray acrylic, a product normally used in photo-finishing. A number of thin coats produces a clear, hard, finish that has good optical quality.

Comparison with the Technique of Bedford (1983)

The technique described in this document was adapted from a technique described by Bedford (1983) of the Lowestoft Laboratory, U.K. A comparison of specifications is outlined in Table 1, and innovations that have proven to be of value are described below.

1. Mold design: (a) It was found that cutting lines could be more conveniently tranferred to resin blocks by etching reference lines in the base of the molds, as opposed to using monofilament chord in the Bedford technique. Mounting lines are similarly scribed in the first resin layer from reference marks on the sides of mold using a straight-edge and probe.

(b) It was found that Bedford's mold design had mounting lines too closely spaced (at 16 mm) to conveniently mount large cod otoliths. A new mold was designed with longer rows spaced 22 mm apart. This design allows large specimens to be placed without conflicting with other rows, and has a more efficient resin volume to otolith ratio (1.6 cc/otolith compared to 1.82 cc/otolith).

2. Releasing agent: Paste-wax was found to be a better releasing agent than silicone grease, as it needs only sparse application and leaves a very smooth finish on the resin block. It can also be used in acrylic molds (where silicone cannot!), and is much less expensive.

3. Cutting fluid: It was found that recycled soluble-oil cutting fluid resulted in blade gumming and overheating. It was messy to use, and required filtration and frequent changing. The system was modified to use unrecycled water, and the above problems were eliminated.

4. Marking sections: It was found that the use of spaghetti in marking cross-sections could be more gainfully employed than in Bedford's technqiue if placed diagonally in the mold in the first resin layer. Top from bottom is then apparent, and the position of the spaghetti section from right to left can then be used to identify the relative sequence of the section. Left from right of the section can also be distinguished by examining the angle at which the spaghetti section traverses the section.

5. Blade specifications: The capability of the hardware at St. Andrews enables full thickness cuts of 12 mm to be made in one pass of the saw, as opposed to six passes with the Lowestoft set-up. This is likely the result of the blade specifications. St. Andrews uses a heavier gauge and recessed design, resulting in both operational time saved and reduced scoring of the sections from repeated exposure to the cutting wheel.

6. Mounting Medium: The use of clear acrylic templates on which to mount sections has the advantage over the use of glass in lower costs, and more versatility in choice of size (custom templates can be cut to any size from large sheets). The use of spray acrylic has proven an expedient method of providing a fast and good quality mounting medium, eliminating the tedious chore of mixing small amounts of resin and applying glass cover slips over the sections.

Comparative Assessment with Traditional Technique

1) Time: Manually preparing 100 pairs of otoliths for viewing requires in excess of 3 hr labour. The old technique entailed a great deal of handling. Otoliths were removed from the envelopes, broken, set in plasticene, oriented for viewing, and then refiled in the correct envelopes for storage. However, the same number of otoliths can be prepared by the new sectioning technique with less than half the time. It should also be noted that sectioned samples are ready for instant re-reading, whereas broken samples require duplicate preparation time.

2) Costs: Material costs for a sectioned sample currently are about $2.00. Material cost for the old technique were negligible (for plasticene and alcohol). Labour requirements however, are substantially less with the new technique, which affords more time for greater production or a higher standard of age data.

3) Viewing Quality: The optical quality of a sectioned sample is judged superior by age readers. Edge type determinations are greatly facilitated due to the contrasting effect of the black resin, and the flat surface of a section renders little distortion from aberrant reflection as is common with

broken specimens. The flat surface of a sawed section also improves assessment of relative ring spacing and interpretation of growth zones. Focussing corrections are minimized because of one focal plane, and depth of field appears to be enhanced. The option of using transmitted light is also possible with sectioned samples.

4) Shelf Life: Broken specimens have been found to lose contrast between growth zones after long-term storage in envelopes (personal observation). Sections are virtually sealed from the air by polyester and acrylic which are chemically inert when cured, and it is anticipated that little deterioration in reading quality will occur with time.

5) Storage and Filing: A legitimate concern in maintaining an inventory of broken samples is the large volume of storage space required, and the associated problems of locating samples filed and stored in large boxes. Sectioned samples require 1/10th the volume that broken otiliths do, and can be filed in small cabinets. All specimens can be located on individual sample units and retrieving a single fish number requires only referring to the ordered header labels on the templates.

6. Impact on Data: The precision of the two methods was assessed by the following study.

Methods: Independent estimates of age were made from pairs of otoliths prepared from both the broken and sectioned technique. One of the otoliths was selected at random for sectioning and the other was broken. Commercial samples from various areas and time were selected for three species for the study. Prior to the results presented here, the readers examined a number of sectioned samples to develop experience and confidence in the new methods. Species-specific orientation of the sections and number of otoliths per strip were established by reader preference.

Results: Comparison of independent estimates of age for each species are given in Tables 2-4 and summarized in Table 5. A total of about 350 otoliths were aged using both methods. Agreement between the two methods ranged from 80% for cod to 85% for pollock and were within 1 year in over 95% of cases. Previous tests of intra-reader agreement for these species suggest a level of 80-90% for experienced readers. No bias was evident between the two methods, although a tendency to underage sections relative to broken otoliths may be present. This comparison also assumes that the two otoliths are mirror images, although readers usually examine both and find that one of the pair provides easier assessment when a specific zone is questionable.

Discussion

Readers expressed preference for the new preparation technique once they had become familiar with the method but noted a requirement to produce high quality sections. Potential problems with the new method include mounting and cutting off center (not through the nucleus), cutting off the correct 90 degree axis and shattered sections caused by less than 1 mm thickness. The first two problems could result in missing the nucleus or other annuli and therefore underestimating the correct age of the otolith. However, these problems are not unique to sections and are easier to control compared to the historical breaking technique. Experience and attention to detail in using this method eliminated problems and produced high quality material. Note must be made that some errors could result in an entire sample being ruined.

Conclusions

Results of this study suggest that the new method of sectioning otoliths of cod, haddock, and pollock provides estimates of age which are within the level of intra-reader agreement. In addition, the saving in preparation time and improved optical quality justify adoption of this method as the standard for age determinaton. Application of the method to other species will require a similar study to assess precision.

References

Bedford, B. C. 1983. A method for preparing sections of large numbers of otoliths embedded in black polyester resin. J. Cons. Int. Explor. Mer. 41: 4-12.

Table 1. Comparison of operational specifications for otolith sectioning of St. Andrews to Lowestoft laboratories.

	Biological Station, St. Andrews, N.B.	Lowestoft Laboratory, U.K.
Cutting machine	Converted surface grinder	Converted surface grinder
Type of blade	Recessed diamond wheel	Flat diamond disc
Arbour washer design	Recessed	Flat
Blade diameter	127 mm	152 mm
Blade thickness	Rim 0.75 mm, Center 0.50 mm	0.25 mm
Diamond abrasive width at rim margin	5 mm	1.6 mm
Abrasive grit	120	100/150
Spindle RPM	2500	3200
Cutting fluid	Water (unrecycled)	Recycled soluble oil
Minimum reliable section thickness	0.5 mm	0.5 mm
Section thickness for large gadoids	1.3 mm	0.5 mm
Number of cuts for 13-mm block	One	Six
Mold construction	Aluminum or acrylic	Aluminum
Mold description	Single block 127 x 152 x 12.5 mm	Double block 76 x 115 x 12.5 mm (ea)
Mounting lines	5 per block spaced 22 mm	6 per block spaced 16 mm
Cutting lines	Etched on sides and bottom to imprint	Monofilament line on reference pins
Mold volume	241 ccs	219 ccs
Otolith capacity	150	120
Volume/otolith ratio	1.6 ccs/otolith	1.8 cc/otolith
Releasing agent	Paste wax	Silicone grease
Otolith orientation	In pairs, proximal sides together, ventral down	Singles, proximal down
Section sequencing and orientation	Diagonal spaghetti piece in bottom resin layer (placed left to right)	Spaghetti parallel to L.H.S. in second layer for orientation only
Mounting template	Acrylic sheets 100 x 175 x 1.6 mm	Glass slides 50 x 76 x 1 mm
Otoliths per template	150 (75 pairs) in 5 rows	30 in 3 rows of 10
Mounting medium	Spray acrylic	Polyester resin and glass cover slip
Labels	Self-adhesive header & row with fish numbers	Self adhesive headers
Production (sawing time)	4 sec/otolith	15 sec/otolith

Table 2. Comparison of estimates of age of cod derived from broken and sectioned otoliths.

		Broken Technique										Totals		
	Age	1	2	3	4	5	6	7	8	9	10+	Broken	Section	Differences
Section Technique	1													
	2													
	3													
	4				14	1						14	15	-1
	5					9	3					10	12	-2
	6						11	1				17	12	+5
	7						3	19	4			21	26	-5
	8								6	1		11	7	+4
	9							1	1	6	1	8	9	-1
	10+									1	3	4	4	0
Totals					14	10	17	21	11	8	4	85	85	

Table 3. Comparison of estimates of age of haddock derived from broken and sectioned otoliths.

		Broken Technique										Totals		
	Age	1	2	3	4	5	6	7	8	9	10+	Broken	Section	Differences
Section Technique	1													
	2		5									6	5	+1
	3		1	10	2							10	13	-3
	4				31	2						34	33	+1
	5				1	23	3					26	27	-1
	6					1	8	2				17	11	+6
	7						6	25	2			30	33	-3
	8							3	8	1		11	12	-1
	9								1	3		4	4	0
	10+										6	6	6	0
Totals			6	10	34	26	17	30	11	4	6	144	144	

Table 4. Comparison of estimates of age of pollock derived from broken and sectioned otoliths.

		Broken Technique										Totals		
	Age	1	2	3	4	5	6	7	8	9	10+	Broken	Section	Differences
Section Technique	1													
	2													
	3			10								10	10	
	4				19	6						22	25	-3
	5				3	46	1					52	50	+2
	6						6					7	6	+1
	7							2	1	1	1	2	5	-3
	8								5	3		7	8	-1
	9								1	4	1	8	6	+2
	10+										10	12	10	+2
Totals				10	22	52	7	2	7	8	12	120	120	

Table 5. Summary of comparison age readings for cod, haddock and pollock using broken and sectioned otoliths. Differences expressed as broken otolith age minus section otolith age.

Species	Number	Percent Agreement	Percent -1	Percent +1	Percent >1
Cod	85	80.0	5.9	12.9	1.2
Haddock	144	82.5	9.1	8.4	0.0
Pollock	120	85.0	3.3	10.0	1.7
Overall	349	82.5	6.1	10.4	1.0

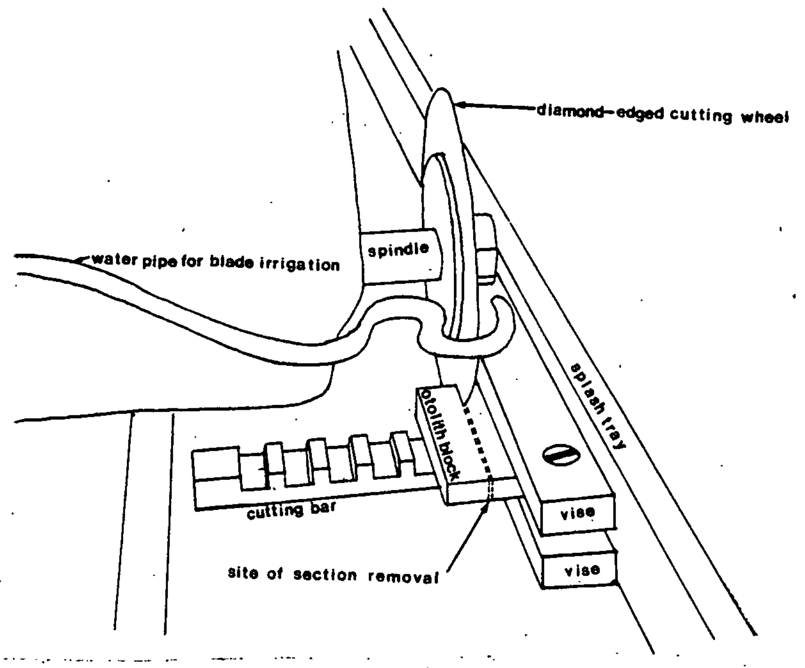

FIGURE 1 THE SECTIONING MACHINE

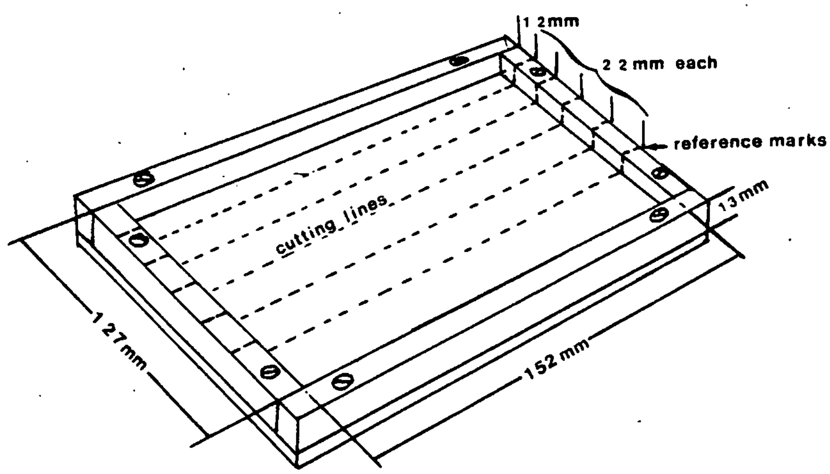

FIGURE 2 MOLD DESIGN FOR LARGE GADOID OTOLITHS