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FURTHER DEVELOPMENT OF THE TECHNIQUE OF PREPARING THIN SECTIONS OF OTOLITHS SET IN BLACK POLYESTER RESIN

B C Bedford

MAFF, Fisheries Laboratory, Lowestoft, NR33 OHT, England

INTRODUCTION

A note to the 1975 Meeting of the Demersal Fish (Northern) Committee by the same author (Bedford, 1975) described a method for preparing thin sections of a number of otoliths at the same time. Previously prepared halves of otoliths were set in a shallow rectangular mould which was then filled with liquid black polyester resin. After the liquid resin had set the mould was placed on a grinding machine and the whole assembly of plastic and otoliths reduced to a thin (less than 1 mm) rectangular sheet. This was carefully removed from the mould and the otoliths read using either transmitted or reflected light.

The method was found to be effective, making the structure of the otoliths more easily visible and reducing reading time very considerably. However the time necessary for the preparation of each individual otolith before setting in the mould and the time taken in grinding were both considerable and a method that could simplify these processes was sought.

A new technique has now been developed in which whole otoliths rather than prepared pieces of otoliths are used with a much deeper mould. The machining process has also been modified allowing the otoliths and plastic to be cut with a diamond edged disc in place of grinding with a carborundum wheel. These processes can be accomplished much more rapidly than with the previous method and results in terms of facility to view the otolith structure are considered to be improved.

The method is particularly suitable for the otoliths of gadoid species, such as cod, haddock, coalfish and whiting, which are normally read using sectioning techniques. Trials have been carried out with the otoliths of other species, including redfish, plaice, hake and horse mackerel, and all have given good results.

DESCRIPTION OF THE MOULD

The mould is made up of a base plate 265 x 125 x 6 mm, two longitudinal strips each 190 x 12.5 x 9 mm and three lateral strips each 100 x 12.5 x 9 mm. These five strips are screwed to the base plate so that when in position as shown in Figure 1a they form the sides of two rectangular moulds each 75 x 100 x 9 mm deep. Across the top face of each of the three lateral strips (marked A, B and C in the figure) six parallel precisely spaced slots are cut. Each slot is 0.50 mm wide and is cut to a depth of 1 mm. The slots are spaced along each strip such that the first is 12.5 mm from the end followed by intervals 15 mm between the remainder. The location of these slots is highly critical and they are cut very precisely by machine.

The material used for making the mould is polyvinyl chloride sheet (PVC) of standard commercial quality. It is important that the base plate be made of a soft but rigid material such as PVC since it may be lightly scored by the diamond disc during machining. The same material has been used for the side strips in all the work done so far and has proved satisfactory. However these pieces are used and removed many times and it is thought likely that metal (probably aluminium) would be more serviceable for long-term use.

PREPARATION OF THE BASE AND MOUNTING THE OTOLITHS

Before assembling the mould, the inward facing edges of the side strips are lightly smeared with silicone grease. This facilitates the removal of the strips after the plastic has set and the casting is complete. With the mould assembled and the sides firmly secured down to the base plate, prepared polyester resin is poured into each half of the mould to a depth of 2.5 to 3 mm to form a base on which the otoliths will be placed (Figure 1b). The material used is supplied as a clear liquid plastic which solidifies after the addition of an appropriate hardener. It is marketed under the brand name 'Plasticraft' by Turner Research Limited of Leeds, UK. At the time of mixing the liquid plastic with its hardener, a few drops of a suitable black dye, manufactured by the same company, are added. When the base has been poured the mould is placed on a level surface for about 2 hours during which time the plastic 'gels' to a thick rubbery consistency. When it is sufficiently set so that the mould may be handled without disturbing the level, a length of monofilament nylon line is threaded through the slots cut into the lateral strips of the mould. A single length of light fishing line is attached to an anchor point (marked X in

Figure 1c) and then threaded through each slot in turn, pulling the line tight until 6 parallel straight lines are formed across the top of the mould as shown in Figure 1c. The loose end is secured at the second anchor point (marked Y in Figure 1c).

The otoliths may now be laid in place in rows on the prepared base using the lines of monofilament as positioning guides. It is important that each otolith be set longitudinally at right-angles to the parallel lines of monofilament with the centre of its nucleus lying directly below the line. For most gadoid species where the inner structure is obscured by the thickness of the otolith, some external reference point can be used to identify the centre of the nucleus and locate it below the line. Two samples of haddock otoliths are shown in place in Figure 1c.

The black polyester resin remains tacky for some hours while it is hardening and otoliths set in the base during this time will remain in place even if the mould is accidentally moved. With otoliths that are flat such as whiting or hake it is unimportant which face is placed downwards onto the base. With otoliths that have a marked curvature it is better to set them with the convex face downwards. This avoids air being trapped underneath the otoliths thereby creating bubbles in the finished solid block. With some large otoliths it is sometimes necessary to break off the extreme ends in order that those in one row do not touch those in the next. A small space (approximately 2-3 mm) should be left between otoliths side by side. For small cod of approximately 30-45 cm total length, 8 or 9 otoliths can be conveniently fitted into a 75 mm row in the mould. For large cod, greater than 90 cm total length, 5 or 6 otoliths constitute a single row. The number of otoliths that can be set in the mould at any one time obviously depends upon the species and size of the fish but it has been found that for samples covering the commercial size range of most gadoids, each 75 x 100 mm section of the mould can accommodate about 50 otoliths.

When otoliths have been set in all the rows a further layer of liquid black resin is poured, filling the mould. Care should be taken to ensure that the plastic fills the space around each otolith and that the mould is filled just to the level of the monofilament lines. When this has been done the mould is left for 24 hours for the plastic to harden.

THE MACHINE AND CUTTING DISCS

The machine used is a standard workshop surface grinder simply adapted to drive a thin (0.25 mm) diamond edged disc instead of a carborundum grinding wheel. A small bench model with the usual facilities of micrometric adjustment to both the cross feed of the table and the vertical feed of the cutting disc has proved adequate for the work. The table, on which the work is held for cutting, has a back and forth traverse of 350 mm and the speed of the cutting disc is 3,000 rpm. The machine is shown in Figure 2.

The cutting discs are 150 mm in diameter and 0.25 mm thick with a 32 mm bore to fit the spindle of the machine. Each face of the disc is encrusted with diamond particles from the outside diameter inward to a depth of 1.6 mm. The overall thickness of this build-up on the edge of the disc and hence the width of the slot cut by it depends upon the grit (ie particle size) of the diamonds. Two different grits have been tested, a fine one of 150/200 giving a cut of 0.56 mm and a coarser one of 100/150 giving a cut of 0.76 mm. Both discs have cut the plastic and otoliths well and have given a good finish. The coarser grit is preferred for regular routine cutting because it clears itself of the fine particles of plastic and otolith more readily.

The adaptation of the machine to take the discs consists simply of two flat stainless steel plates each 120 mm diameter and 6 mm thick, which fit one on either side of the cutting disc on the spindle of the machine. These plates provide a rigid support for the cutting disc while permitting it to cut to a depth of 16 mm. The discs used are obtained from Messrs Treifus Ltd, Crawley, Sussex, UK. The plates and disc are shown in Figure 3.

CUTTING AND MOUNTING THE OTOLITH SECTIONS

When the plastic has hardened completely the monofilament line is stripped from the mould and the side strips are removed leaving two rectangular islands of solid black plastic attached to the base only by adhesion. The base plate is then clamped to the table of the machine for cutting by the diamond disc. The location of the two blocks of plastic relative to the cutting disc is extremely important and the base plate must be positioned so that the lines on which the otoliths lie are exactly parallel to the back and forth traverse of the table. A simple jig arrangement permanently attached to the table of the

machine allows this to be done quickly and accurately each time. To locate the position of the rows of otoliths in the lateral plane the edge of the diamond disc is carefully offered up to the vertical edge of the blocks until a very light contact is made along the entire length of the two blocks. The micrometric adjustment of the crossfeed allows this to be done very precisely and with a little practice the whole operation of setting up can be carried out very quickly.

Once the base plate is securely clamped to the table in the correct position (Figure 3) the first cut can be made along the first row of otoliths. This row is 12.5 mm into the block from its edge so, after raising the disc to clear the surface of the block, the micrometric cross feed is used to move the whole table 12.5 mm and place the row directly beneath the disc. The thickness of the block is too great (approximately 9 mm) for it to be cut with a single heavy cut and a series of five or six light cuts each of about 1.5 mm is made, the work being moved back and forth below the disc for each cut. Throughout the cutting operation the entire work surface is flooded with a soluble oil coolant permitting the machining to be done rapidly without damage through overheating to either the otoliths or the pastic (see Figure 5). Using the micrometric vertical feed the final cut is made very precisely so that the cutting disc just touches the PVC base plate along the entire length of the plastic blocks. The two small sections of plastic that have been cut, one from each block can now be removed from the base plate. This is done by pressing the sharp edge of a knife or scalpel blade under one corner and gently prising the piece away from the base plate.

This exposes the otoliths embedded in the section of the two blocks still attached to the base plate. (Figure 4) A second cut is now made to remove a thin slice from this exposed end (Figure 5). This can be of any thickness above a minimum of about 0.25 mm. Experience has shown that a slice of between 0.65 mm and 0.75 mm combines good otolith visibility characteristics with sufficient robustness to permit easy handling. Again using the crossfeed adjustment of the table the work is moved a further 1.35 mm under the diamond disc and the cutting process is carried out again down to the base plate. Using the coarse grit diamond disc

(0.76 mm slot width) leaves a thin slice of approximately 0.69 mm detached from the main block and attached only to the base plate. This is prised loose and placed on a glass slide 75 x 50 mm. To avoid damage during removal of the slice from the base a sound technique is to use the small block already removed to apply a uniform gentle sideways pressure on the slice before prising loose with the sharp blade (Figure 6).

With the cutting of the first row complete the crossfeed of the machine is advanced a further 13.65 mm (spacing between rows of otoliths is 15 mm) to position the second row directly below the disc. The process of two precisely spaced cuts is then repeated for this and subsequent rows until all six have been cut and the slices removed and placed on glass slides. Each half of the mould produces 6 slices, each 75 x 9 mm and the 12 from the whole mould can be conveniently arranged on four of the glass slides (75 x 50 mm).

Finally the slices are permanently fixed to the glass slides again using polyester resin. After the three slices have been set in position a thin layer of clear polyester resin (with the appropriate hardener added) is poured to cover the slices and the remainder of the slide. While this is still liquid a thin glass cover slip of a slightly smaller size (70 x 45 mm) is floated on to the surface. The finished slide is then left for a further period for the plastic to harden after which the otoliths are ready to be read.

With the monthly samples of otoliths from commercial catches that are regularly processed by this method at Lowestoft, the details of the samples are etched on the glass cover slip with a diamond scriber thereby providing a permanent record. A finished slide is shown in Figure 7.

CONCLUSION

This new method of preparation of large numbers of otoliths at one time is thought to be a considerable improvement over the method previously described by the author. It is considered that it also offers advantages over conventional methods presently used for the regular reading of large numbers of otoliths of some gadoids and may well have useful application with other species. These advantages are:

1 The skills needed for setting up the otoliths, operating the machine and preparing the slides can be easily learned and carried out by laboratory technicians, thus eliminating the non-productive time spent by the otolith reader in preparing sections one at a time.

2 The facility to view the otolith structure is at least as good as by conventional methods and in many cases is considered to be improved. A feature of this is a more accurate definition of the nature of the edge, made possible by the contrast of the otolith with the surrounding black material.

3 Comparison of one otolith with another either on the same slide or on another is much simpler than methods involving the mounting of single individual otoliths.

4 Either transmitted or reflected light may be used with equal facility and changing from one to another is easily effected.

5 Slide mounted otolith sections whether lit by transmitted or reflected light lend themselves readily to viewing by closed-circuit TV methods and so make teaching and demonstration simpler.

6 The space needed for otolith storage is greatly reduced probably by a factor of as much as ten times. Standard storage cabinets obtained from a commercial laboratory equipment supplier have been successfully used.

REFERENCE

BEDFORD, B. C., 1975. A new technique for reading otoliths. ICES CM 1975/F:36, 3 pp (mimeo).

The reference to proprietary products in this paper should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

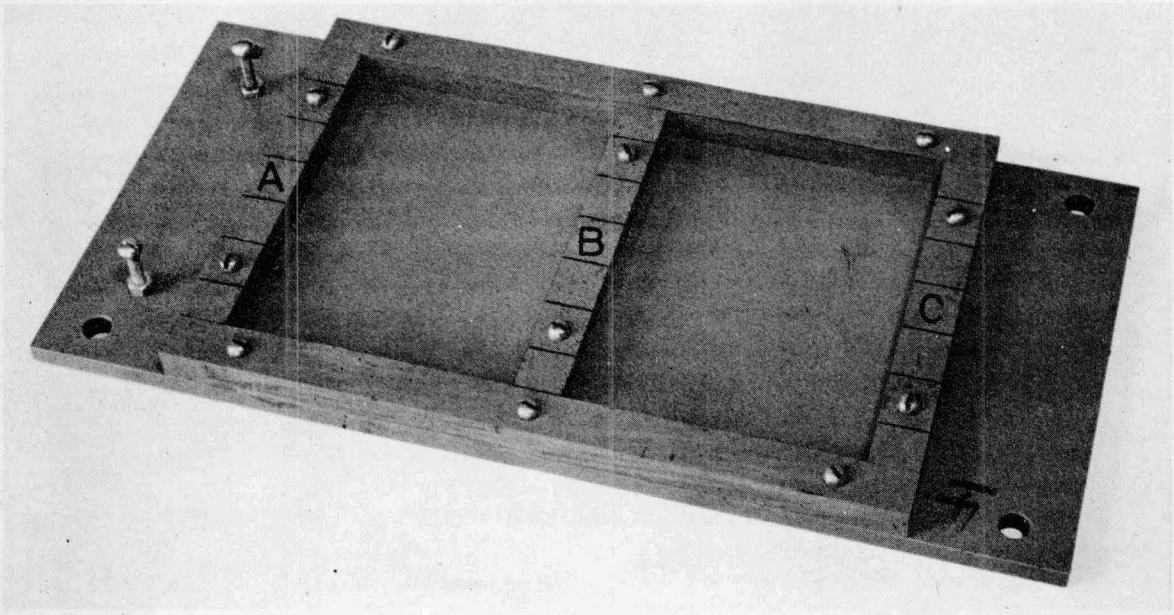


Fig.1a. The mould empty.

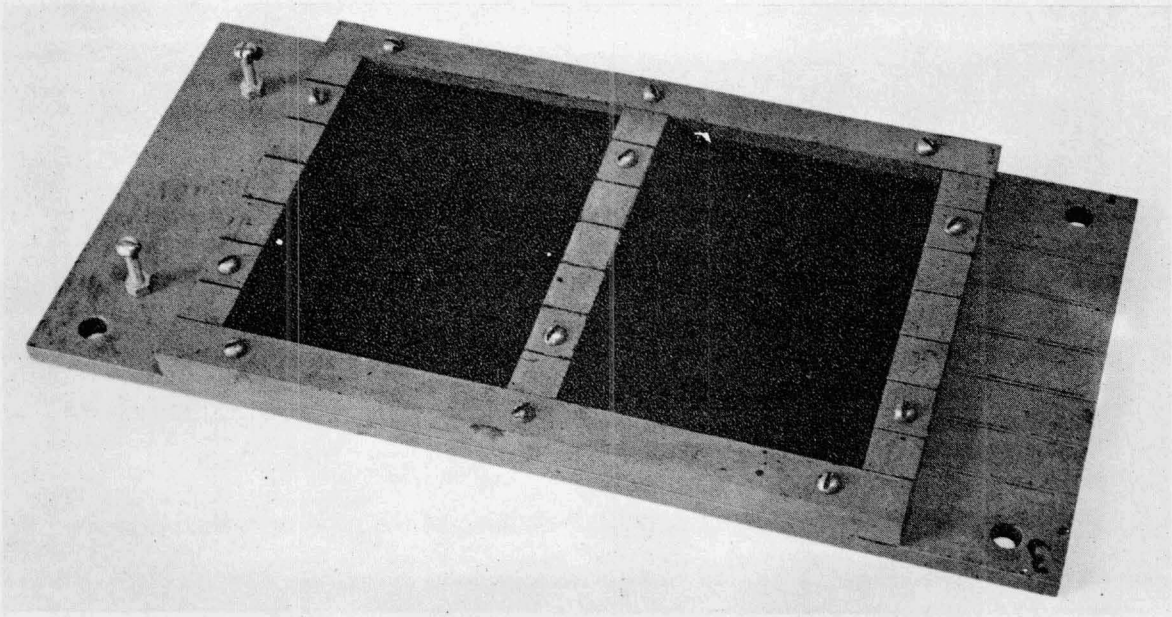


Fig.1b. The mould with bed prepared.

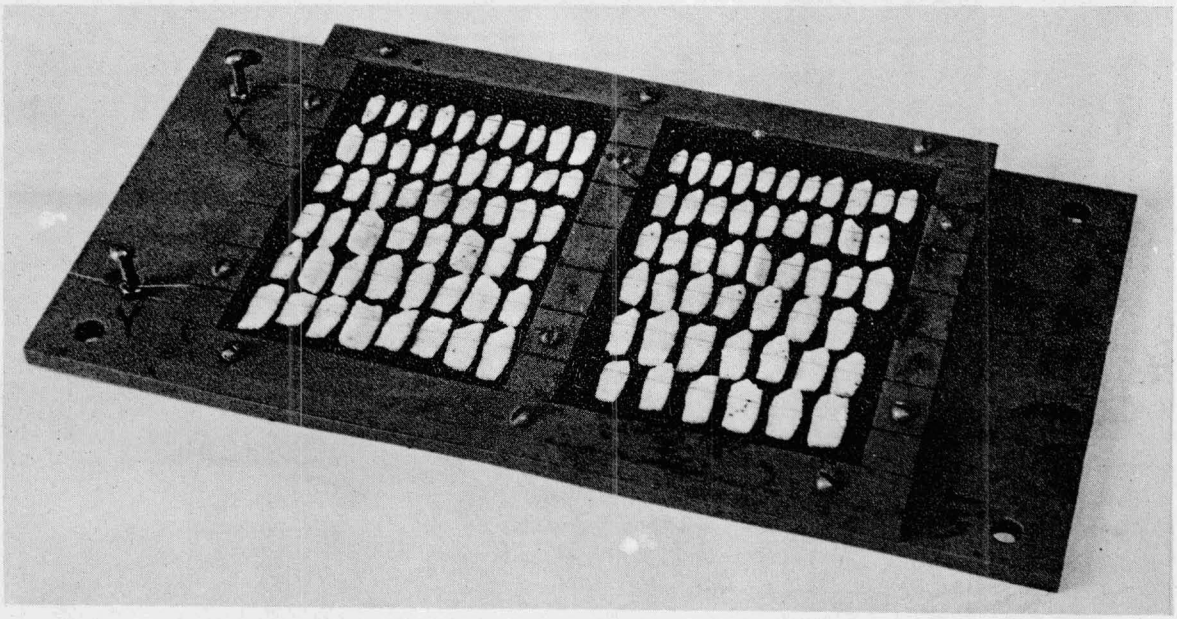


Fig.1c. The mould with otoliths set in position

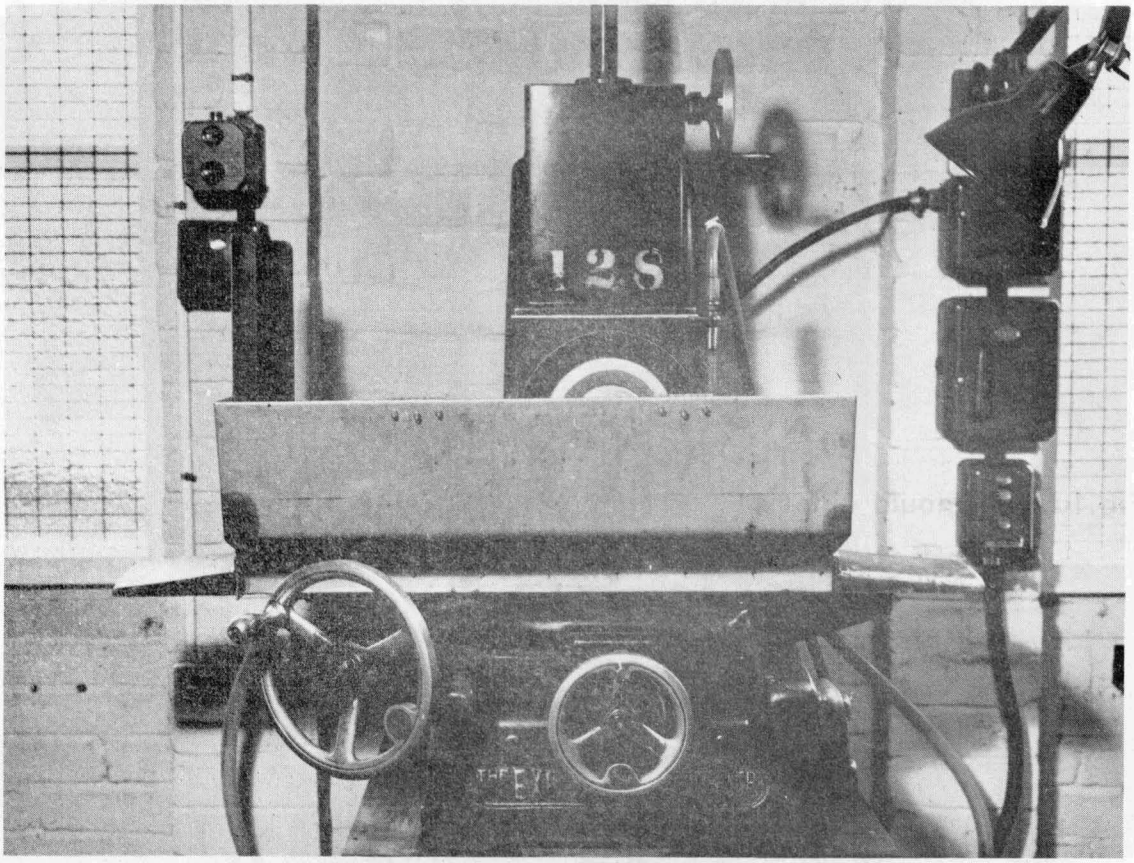


Fig.2. The machine, general view. N.B. The anti-splash guard shown partially covering the cutting wheel is removed in subsequent photographs.

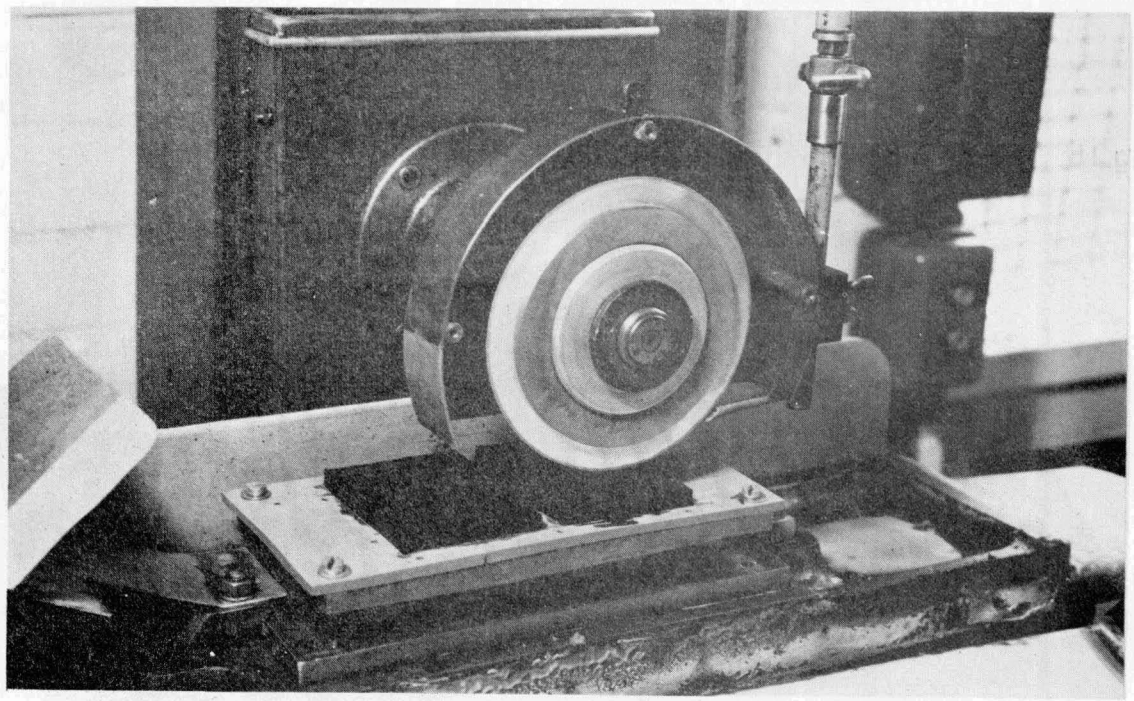


Fig. 3 The mould in position ready for the first cut.

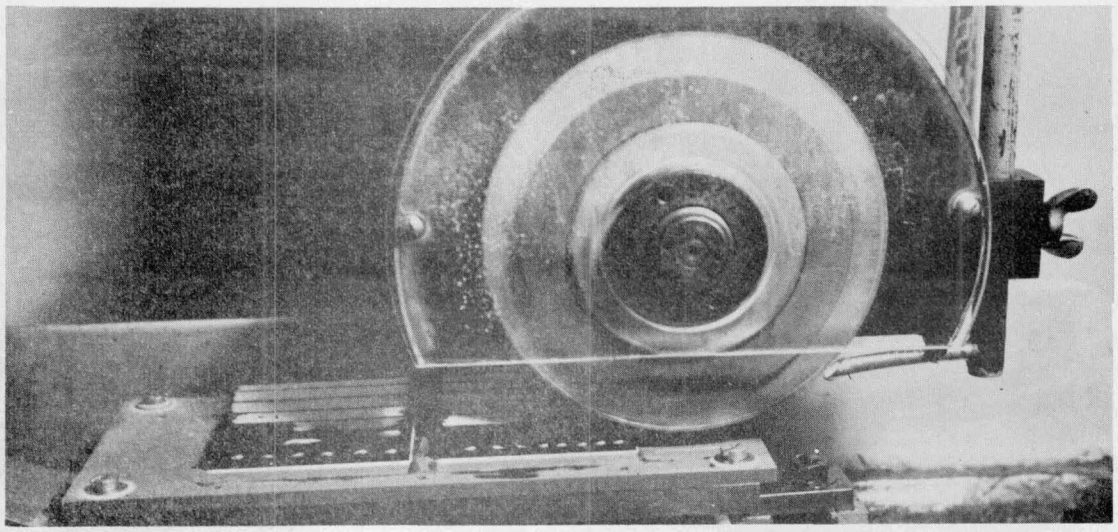


Fig.4. The mould after the first cut has been made and the first section removed.

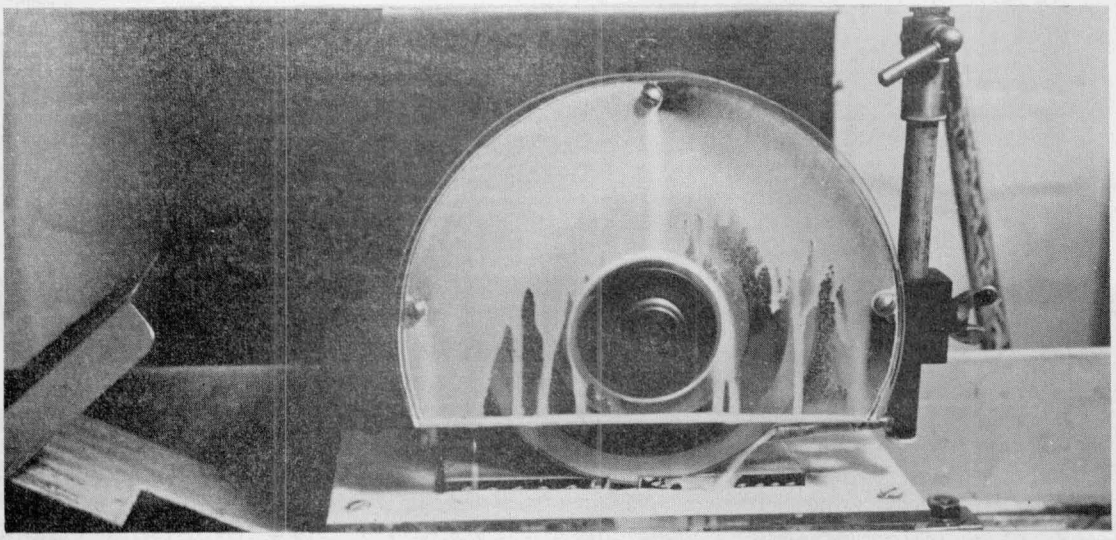


Fig.5. Machining in progress, cutting the first slice.

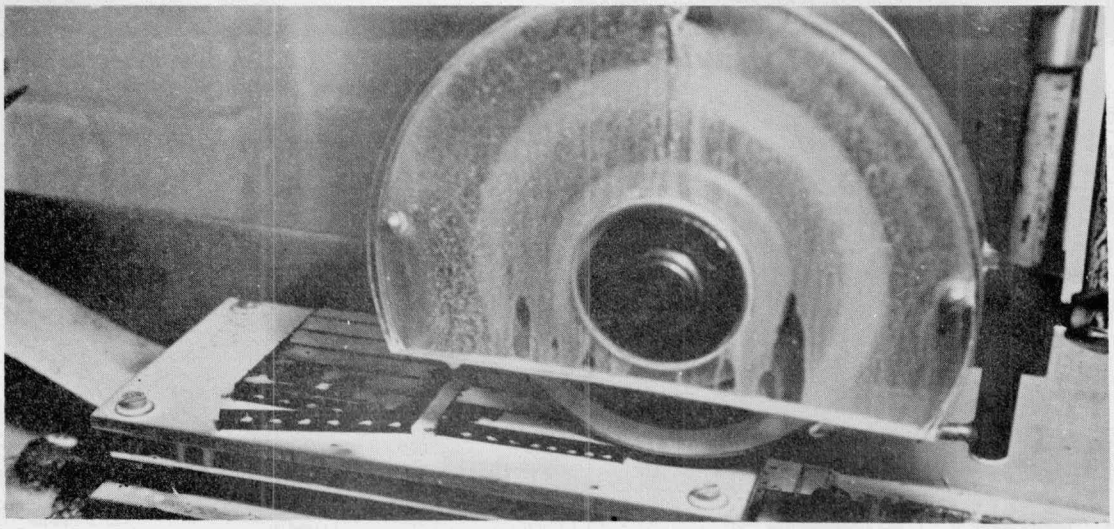
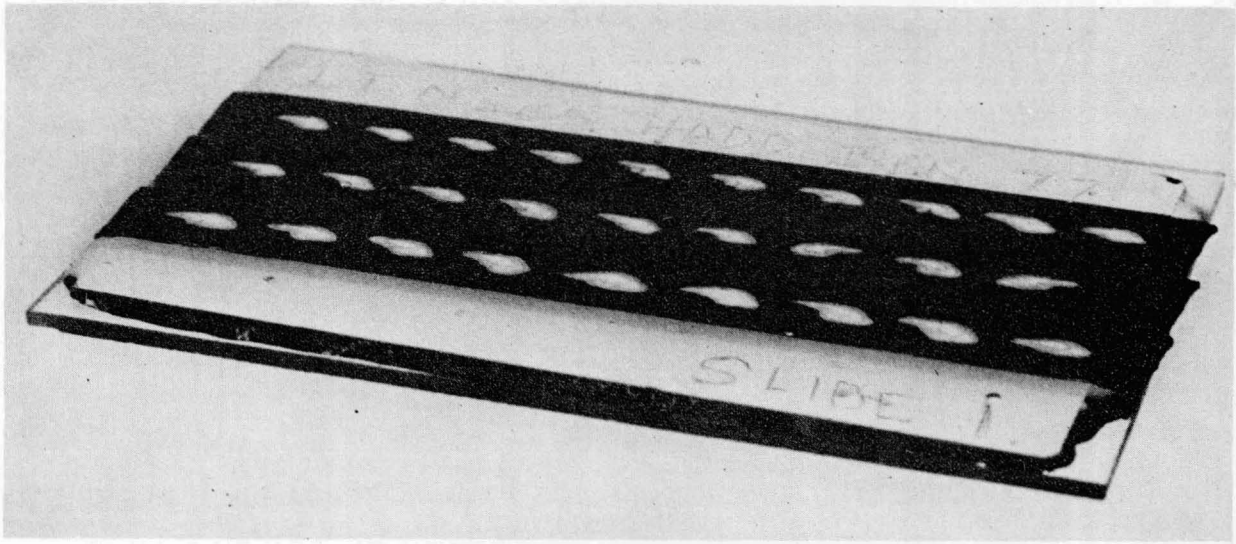


Fig.6. After completion of the second cut showing the slice detached from the main block.



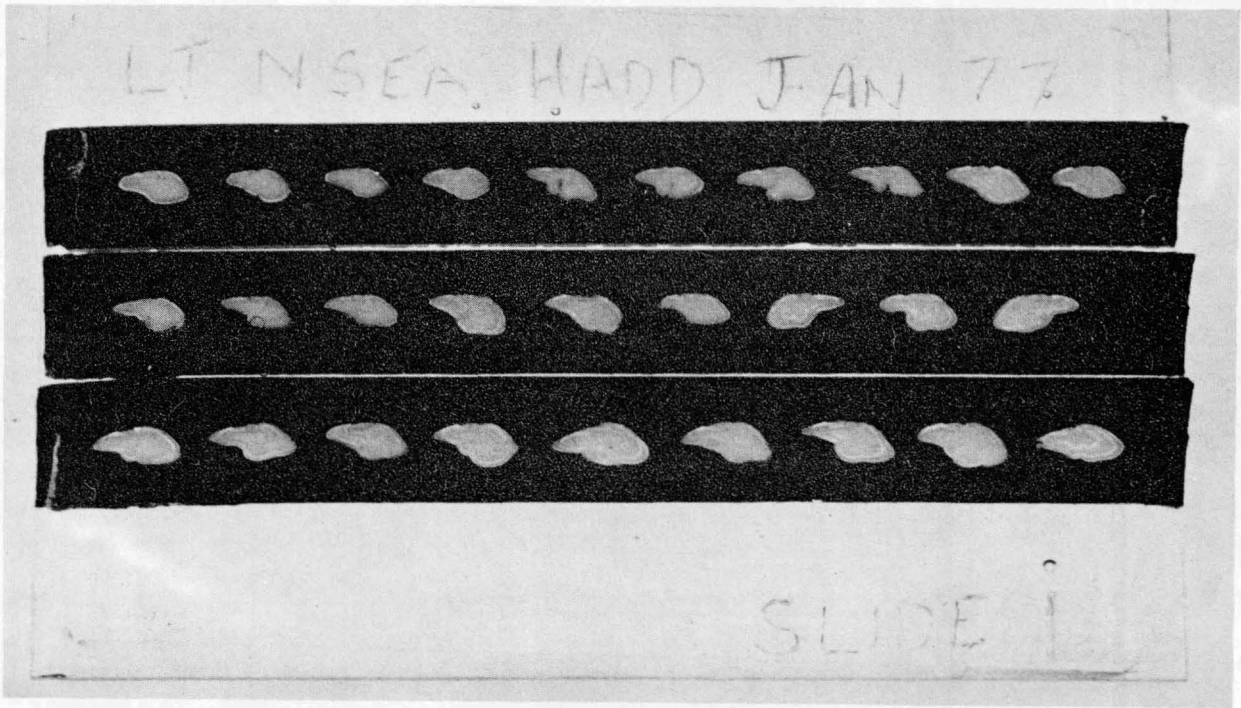


Fig 7. Two views of a prepared 75x50mm slide ready for reading.