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A guide to good otolith cutting

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Fish for the future

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A guide to good otolith cutting

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Abstract

This is a guide for embedding and sectioning fish otoliths. It is based on many years of otolith sectioning experience and is written as a recipe book for novice and expert alike. Included is a method for the enhancement of the clarity of small otoliths for improved sectioning, development of custom made tools, as well as a list of do's and don'ts and hints to make the job easier. Use of this guide will assist in the most reliable interpretation of an otolith's internal structure, thereby improving ageing studies in general.

1.0 Introduction

Otoliths are essential to both fish and fish researchers. To the fish these small calcareous structures play a central role in sensing balance, motion, gravity and sound (Lowenstein 1971). To the researcher they are a recorder of chronological information that can be used to trace back a fish's life history with a level of detail unmatched by any other structure within any other vertebrate (Campana and Thorrold 2001). This is largely because of their unique properties of continuous, lifelong growth and immunity to resorption (Campana and Neilson 1985). As such, over the past decade there has been growing interest in the use of otolith chemistry to reconstruct the environmental history, ontogenetic patterns of movement and stock discrimination of fish (e.g. Edmonds *et al.,* 1991, Secor 1992). The most common function of otoliths nevertheless continues to be as a tool for determining the age of individual fish in studies of growth and mortality (Campana and Thorrold 2001, Secor *et al.,* 1995).

Otoliths therefore have a key role in the sustainable management of fish species, although their interpretation is generally a subjective and unreliable science. For instance, in a comparison among several experienced otolith readers the number of annuli counted in the sectioned otoliths of tailor (*Pomatomous saltrix*) varied by up to five years for fish that had a maximum age of about seven years (S. Ayvazian, W.A. Marine Research Laboratories, *pers. comm.*). These differences were largely due to an unfamiliarity with the otoliths of that species and would decrease with further experience. However such errors may be compounded by inadequate preparation of the otolith in the first place – a point of particular importance where sectioned otoliths are used because of the lengthy process involved, but one which is often given too little emphasis. With this in mind, the aim of this report is to provide a step-by-step guide to the preparation of otolith sections, using methods that have been developed and proven on a range of temperate and tropical marine species including coral trout (*Plectropomus maculatus*), pink snapper (*Pagrus auratus*), Spanish mackerel (*Scomberomorus commerson*), gold-banded jobfish (*Pristipomoides multidens*), spangled emperor (*Lethrinus nebulosus*), tailor (*Pomatomous saltrix*), and red emperor (*Lutjanus sebae)*. Whilst the internal structure and readability of fish otoliths may vary considerably, the methods described here should be broadly applicable to the otoliths of other fish species also.

2.0 Methods

2.1 Basic principles

The aim of sectioning otoliths should be to obtain the best possible interpretation of the internal structure of the otolith. In achieving this, the alignment procedure described here may take a little longer but will deliver better results. With a more difficult species, such as *Pristipomoides multidens*, it can make the difference between the section being readable or otherwise.

The most common error of sectioning otoliths is an incorrect angle of cut. This is usually the result of an accumulation of small errors. In the sectioned otolith, the best annuli (banding) readability is obtained by cutting perpendicular through the annuli such that they are as narrow and well defined as possible. If the cutting angle is not correct, the banding will appear diffused, often interfering with the increments next to it and making reading difficult. A useful analogy is looking through open vertical blinds or louvres. The louvre directly in line with the viewer, exhibits a narrow profile. Any angle of view away from this orientation and the view of the louvre blades become broader. The way to achieve this narrow profile is to cut the otolith at 90 degrees to that part of the sulcus acusticus over the core of the otolith (Figure 1). **Do not** line up the anterior and posterior points of the otolith as a guide to the cutting angle (as is frequently done), since this may be more than 10 degrees from the optimum angle in some species.

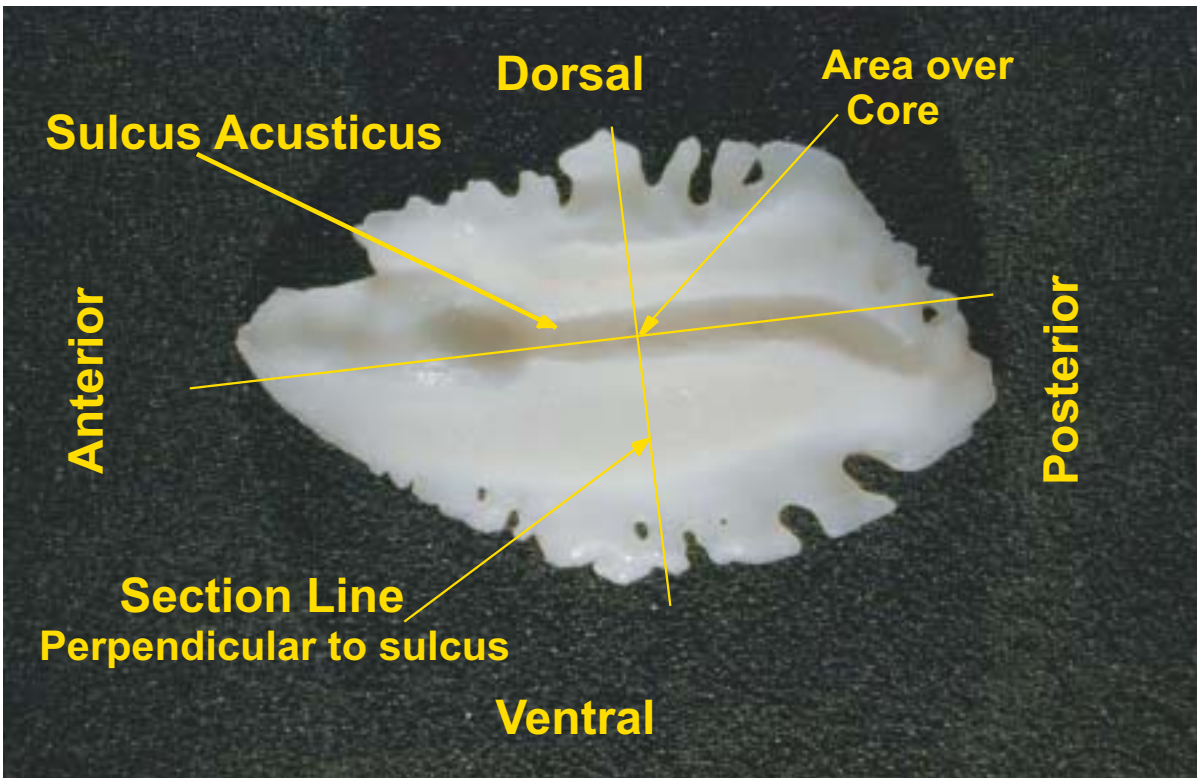
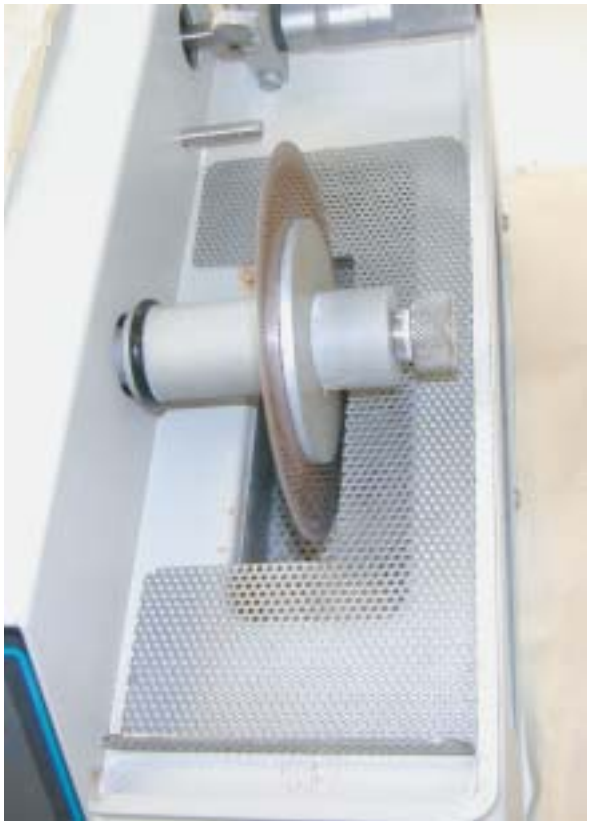


Figure 1. An otolith from a Gold-banded Jobfish (*Pristipomoides multidens*) with the optimal cutting angle shown.

2.2 Equipment used

- Dissecting microscope with a diascopic illuminator base
- Cold light source with a twin branch light guide
- Low speed saw with diamond wafering blade
- Computer with spread sheet
- Vernier callipers – preferably digital
- Moulds for embedding otoliths (Figure 2)
- Release agent
- Epoxy resin
- Casting resin
- Syringe
- Disposable pipettes (3ml)
- Pop sticks
- Fine wet and dry paper
- Perforated guard (custom made) (Figure 3, Appendix 1)
- Alignment block (custom made) (Figure 4, Appendix 2)
- Alignment jig (custom made) (Figure 5, Appendix 3)
- Magnifying glass
- Small scissors
- Fine tweezers
- 2% HCl
- Water containers
- Detergent
- Low linting tissues
- Fine permanent marker (0.4mm)
- Pencil
- Frosted slides
- Cover slips

Figure 2. Ice block tray used as moulds for embedding otoliths.



Figures 3. Perforated guard.

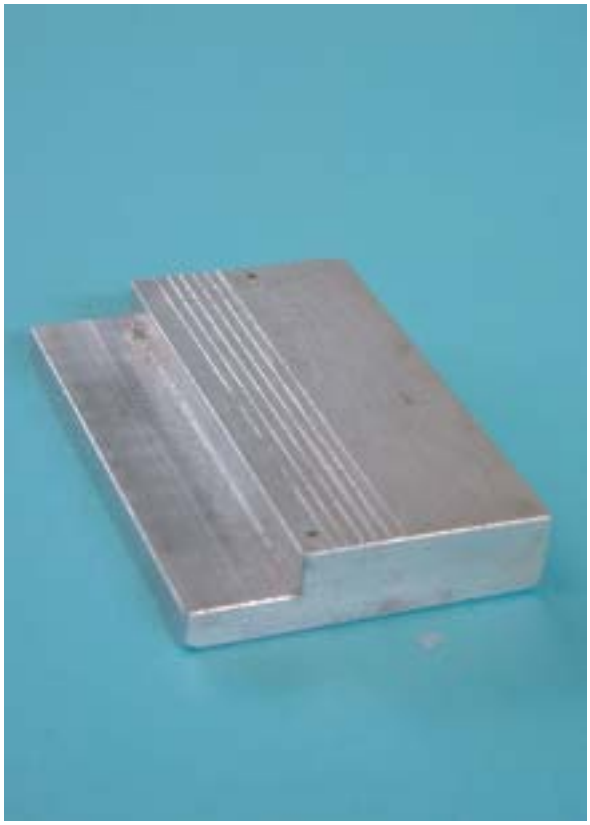


Figure 4. Alignment block.

2.3 Embedding the otolith

2.3.1 Moulds

Plastic ice block trays (Figure 2) available from the local supermarket make good, low cost and easily accessible moulds. These trays have 14 sections of approximately 16 ml volume each. However, they will eventually buckle and should then be thrown out or weighted down to straighten them when in use. Avoid white ice block trays as the lack of contrast makes placement of the otolith difficult in these.

2.3.2 Release agent

To facilitate easy removal of the embedded otolith, the mould should first be coated with a release agent prior to pouring the resin bases. This can be any readily available CRC, WD40 or Silicone Spray type of product used to free rusted parts etc. A pressure pack is preferred for ease of use. The release agent coating must be kept as light as possible so as not to interfere with the second resin pouring. After lightly spraying the moulds, leave the trays upside down on some paper towel for a while to drain off any excess. Before turning the trays to their upright position, lift them up and wipe around underneath so that any accumulated excess will not run back into the mould when flipped over.

You have used too much release agent if it wells up onto the resin surface after pouring the bases. Too much of some release agents will react with the resin and make the bases go opaque.

2.3.3 Resins

There are numerous resins that can be used for embedding otoliths including:

Casting Resin: this is a reasonably hard resin. It can be used for embedding otoliths but is generally reserved for mounting otolith sections and cover slips onto slides. Because it sets hard and brittle it is more difficult to trim the cut section before mounting them onto slides and it takes longer to cut through. Casting resin remains clear when set.

General Purpose Low Viscosity Epoxy Resin FR 251: this is a two to one mix, easy and fast to cut. Used only for embedding otoliths. Not suitable for mounting sections onto slides as it discolours to a light honey colour when set.

2.3.4 Bases

When mixing resin, follow the manufacturer's instructions. In particular, make sure that the catalyst is thoroughly mixed through the resin but be careful not to aerate the mix, as air bubbles will not readily dissipate out of the mix and can interfere with lining up the otolith later for marking. They will also weaken the mix. Pour enough resin into the mould to just cover the bottom. Any more than this is a waste and extends cutting time. Use a suitable size syringe for this process, 20 to 60 ml, depending on size and number of moulds you are using. After having poured a series of bases, check that none of the resin has retracted from the corners of the moulds. If so, add a little more resin. As a guide, a little less than 1 ml is enough to cover the bases in the moulds. It is essential that the trays remain flat and are placed on a level surface while the resin is setting. If this is not achieved the resin will cure in a wedge shape and introduce unwanted angles later when placing the otolith. Let the resin bases harden before proceeding further so that the otolith rests flat and can easily be manoeuvred when placed later.

2.3.5 Sample identification

An easy way to add identification numbers to the otolith sample is to create them in an Excel spreadsheet. If names and numbers are reduced to a font size of 6, and the columns and rows adjusted accordingly, they will easily fit into the mould alongside the otolith. Alternatively write the identification numbers on a narrow strip of paper for insertion into the mould. Use a pencil, anything else will bleed into the resin and become difficult to read. To prevent sample identification mistakes when there is more than one person involved in producing otolith blocks, make sure that the last identification number used is displayed in a prominent place for reference (eg on a white board).

2.3.6 Embedding otoliths

Place the identification number in the mould and to the edge of the base so that it will not be obscured by the otolith. Add the otolith, **sulcus downwards**. Positioning is not important at this time.

2.3.7 Large otolith

Otoliths that are almost as large as the mould that they are going into, are best positioned if either one of the anterior or posterior points are cut back. Because of their brittle nature otoliths shatter easily, so care has to be taken not to cut close to the core. Removal of about

one quarter of the otolith is usually sufficient and can be done by using surgical bone cutters, electrical side cutters or a grinding wheel. This shortening is necessary to allow the core of the otolith to be positioned as far to one end of the mould as possible so that the resin block can later be inserted far enough into the chuck for a good and level grip. Otherwise unwanted cutting angles will be introduced. If the core is not far enough out from the chuck when good seating is achieved, the inner flange for the cutting blade will interfere with the chuck when trying to access the core.

Add resin to a level that only just covers the anterior and posterior tips of the otolith. When pouring the resin, do so from one end of the otolith so that air does not become trapped in the sulcus. Air bubbles formed against the otolith will weaken the resin's grip and can cause part of the otolith section to crack or fall out when cut. Otolith orientation and placement in the mould should be such that the sulcus section near the core is aligned longitudinally and parallel to the sides of the mould and that the otolith is placed in the centre with either anterior or posterior or cut off end almost against one end of the mould. Check that the sample identification is still to one side and that the number is not obscured by the otolith. Finally, press down lightly on the otolith at the core so that the centre part of the sulcus is flat and in touch with the base. A sharpened pop stick or similar works fine for manoeuvring otolith and identification tags. Correct otolith orientation and placement pays dividends later when the block needs to be inserted into the chuck. If the otolith placement is too far out of line, the block will have to be inserted into the chuck at too great an angle to align properly and will make cutting difficult. It is important to let the resin cure with the moulds in a flat and horizontal position. This produces even blocks and reduces parallax errors later when marking them for the cutting angle.

2.3.8 Removal from moulds

Once set, otolith blocks are easily removed by slightly twisting the mould. Older moulds may need some assistance by applying a little thumb pressure against the back of the individual blocks whilst upside down on a bench.

2.4 Marking otolith blocks for cutting

This is a three-stage process.

2.4.1 Marking the core

1. Marking the core is best done on a dissecting microscope with a diascopic illuminator base. With transmitted light (coming through from underneath), and the otolith block sitting flat on the stage (**sulcus down**), the core is usually easily visible. Mark this spot with a fine permanent pen, eg Artline 250. It is important to accurately mark the core, as this is the target of all your cutting effort later. Do not hold the otolith block up to the light to mark the core, this is too uncontrolled and will introduce parallax errors.

2.4.2 Marking the anterior and posterior ends

2. The object of the second stage of marking, and probably the most difficult, is to mark the anterior and posterior ends of the otolith block in direct line with that straight part of the sulcus that runs across the core. It is important to achieve this

correctly because a line at right angles to this will later become your cutting angle. Draw a straight line (100 mm will suffice) on the work bench, a piece of paper or card. Place the otolith block longitudinally and upside down (**sulcus up**) on top of this line. The objective is to line up that straight middle part of the sulcus which lays across the core with the drawn line, as viewed from above. To enhance the visibility of the sulcus, illuminate the otolith from both sides with the twin light guides right up against the sides of the otolith block. Manoeuvre the lights up or down slightly to obtain the best relief of the sulcus. Viewing from directly above, line up that part of the sulcus above the core with the drawn line, hold the otolith block in place and mark both ends of the block where the block and the line meet. To assist with this lining up procedure, especially with smaller otoliths, use a freestanding 90 mm magnifying glass (Figure 5).

Alignment Jig

An alignment jig can be used to facilitate marking of the block (Figure 5, Appendix 3). This consists of a small mirror (approximately 75 mm x 150 mm) glued to a wooden base with two 30 mm raised ends. String a light piece of fishing line (2 kg breaking strain) from the middle of one raised end to the other, holding the line in place with a screw at each end. Place the otolith block (**sulcus up**) on the mirror, directly under the line (Figure 6) so that the line longitudinally dissects the centre part of the sulcus above the core. Hold the block in place and mark both ends of the otolith block at mirror level when and where the line and its mirror image are as one.

Figure 5. Alignment jig and magnifying glass.

Figure 6. Initial block alignment showing sulcus alignment and end marks.

2.4.3 Obtaining the cutting angle

3. To obtain the correct cutting angle, an imaginary line between the marks obtained on the ends of the otolith block in section two, has to be bisected at right angles. This line then becomes the guide for lining up the otolith block in the chuck. To achieve this, draw a right angled cross on an appropriate flat surface (bench, paper or card). Place the otolith block on the cross (**sulcus down**) with the previously marked core (stage 1) close to or on the intersection of the marked lines. Line up the marks made on the ends of the block (stage 2) with the north – south line of the cross (Figure 7) and place a small straight edged ruler (a large 50 x 75 mm glass slide is good for this because of its transparency) across the block in line parallel to the east – west line and draw a line across the block with a fine permanent pen. Do not obscure the previously marked core as this is required for lining up with the saw blade.

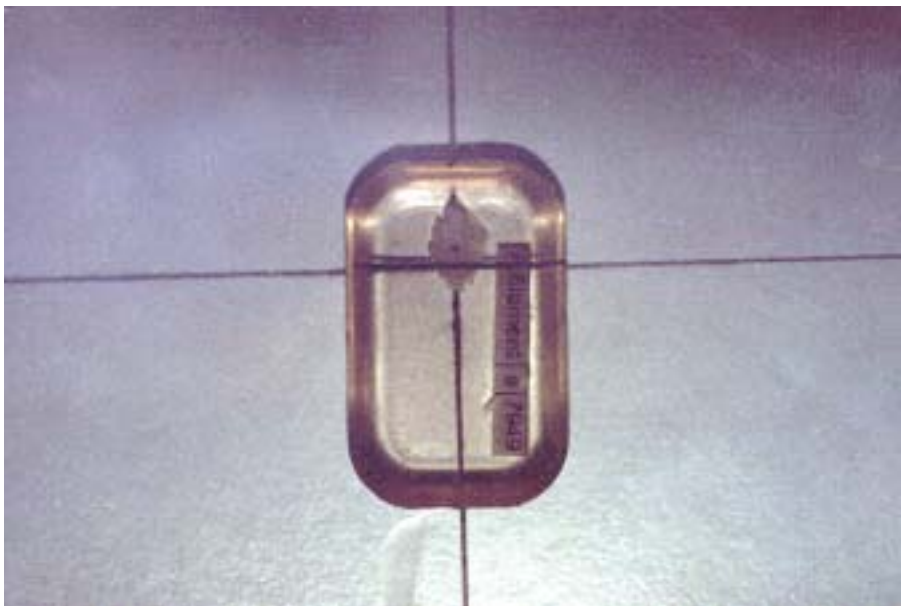


Figure 7. Alignment for marking the cutting line.

2.5 Enhancement of the clarity of small otoliths and the rough surface of embedded blocks

With small (generally < 15 mm in length) or very thin otoliths it is difficult to see the relief of the sulcus when embedded in resin, thus making the alignment preparation for cutting difficult. To overcome this difficulty, first mark the core (**sulcus down**), then enhance the centre section of the sulcus by drawing along the straight middle section of its length (Figure 8) with a sharp pencil before embedding the otolith. This is best done under the microscope.

Figure 8. Small otolith marked prior to embedding.

Moulds that have been in use for some time may produce blocks with roughened surfaces. This makes it difficult to see the relief of the sulcus and difficult to implement the line up procedure, especially in small otoliths. Good transparency through to the sulcus can be regained by adding a few drops of water to the surface of the block, forming a lens above the sulcus.

2.6 Sectioning the embedded otolith

The comments in this section apply to the use of a Buehler Isomet Low Speed Saw using a 102 mm x 0.3 mm Diamond Wafering Blade, although the principles should be valid for all saws.

Always cut a small trial batch of otoliths for each species to gauge the appropriate cutting thickness for good readability. Section thickness will vary from 120 to 300 microns, depending on the translucency of the otolith and the definition achieved from the annuli. As a starting point aim for 200 micron sections, although ideally the best thickness should be determined by sectioning left and right otolith pairs at different thicknesses and comparing readability. In some species, such as *Scomberomorus commerson*, it may also be necessary to cut three sections at differing thicknesses because the optimum varies between otoliths.

There are a few important points and principles to observe before the cutting can begin:

2.6.1 Holding the otolith block in the chuck

The otolith block is inserted between the chuck jaws with the bottom of the block (**sulcus down**) resting on the main body of the chuck. Care must be taken to insert the block far enough into the chuck so that the curvature at the base of the block is further than halfway into the chuck, and preferably extends right through the chuck. If this is not done, the block will have a tendency to tilt up (Figure 9) as you tighten the clamping bar down onto the otolith block. The bottom of the block must sit flat on the chuck otherwise you will introduce unwanted angles when cutting the sections. Cutting access to the core should be achievable and therefore not present a problem if the otolith has been moved right up to one end of the mould when embedding. Settle the block into the chuck so that the line drawn across the block is approximately parallel to the chuck face.

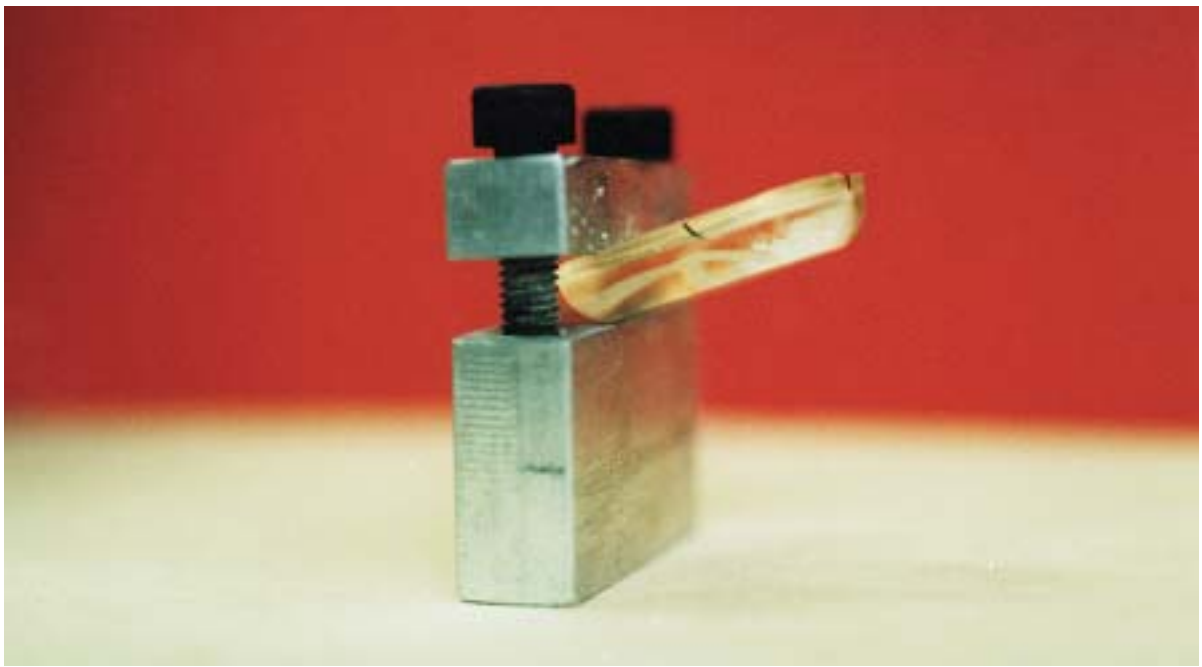


Figure 9. Consequences of incorrect mounting, showing otolith block tilt because the block had not been inserted far enough into the chuck.

2.6.2 Tightening the clamping bar onto the otolith block

Care must be taken to settle the chuck clamping bar onto the block before the clamping bolts are tightened. Both bolt heads **must** be clear of the chuck clamping bar when holding the bar down onto the block. This is best done with the thumb on top and in the middle of the bar with the other fingers supporting the main body of the chuck from underneath. If the bolt heads are not clear of the bar when settling it into place there is a tendency for one side of the otolith block to lift off the main body of the chuck (Figure 10). This makes the otolith block unstable and can cause problems when cutting. Tighten the bar finger tight only.

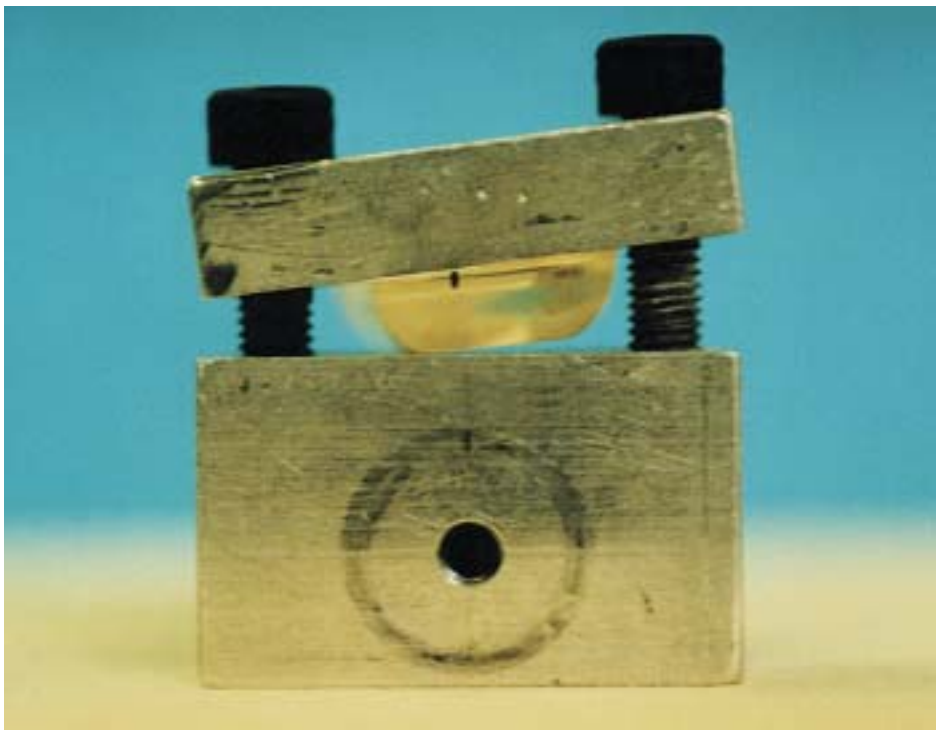


Figure 10. Consequences of incorrect clamping, showing a gap between the otolith block and the main body of the chuck.

2.6.3 Lining up the otolith for cutting may be facilitated in two ways:

1. Draw a series of 6 to 10 parallel lines, approximately 70 mm long and 2 mm apart, on a suitable flat surface such as the bench top, paper or card. Place the base of the chuck against the first line. View the block from the top and move it (without moving the chuck) until the cross section line previously drawn on the block is parallel to the lines below it. Check that the chuck is still in line with the first line, then tighten the cross bar bolts. **Re-check alignment after tightening**.

2. **Alternatively**, you can manufacture an alignment block (Figure 4, Appendix 2) from a 50 mm wide x 12 mm deep x 70 mm long aluminium bar, with a 6 mm deep x 15 mm wide recess machined out of one edge and 6 fine parallel lines, 2 mm apart, engraved on top parallel to the recess. These lines can be coloured in with a permanent marker pen if their visibility becomes a problem. Place the base of the chuck into the recess and hold it against the step. Line up the otolith block's cross section line with the parallel lines on the alignment block and

tighten the chuck's clamping bolts (Figure 11). **Re-check alignment after tightening**. The alignment block is easier to use because it will not allow the chuck to move around as is possible with the procedure above. However, never tighten the chuck clamping bolts while the chuck is attached to the saw's support arm. This will lead to a bent support arm and invalidate the otolith lining up procedure.

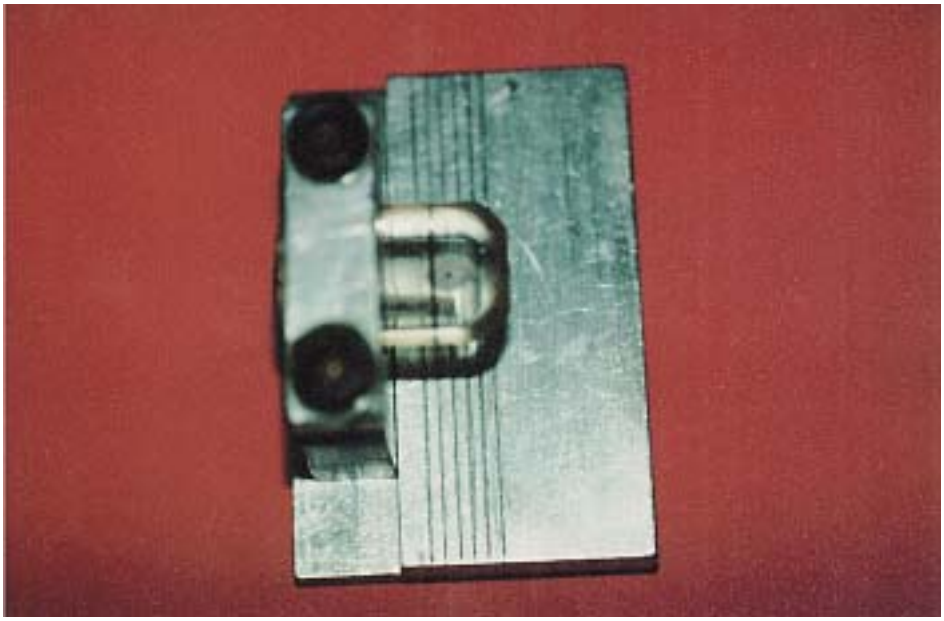


Figure 11. Otolith block in the chuck, showing alignment with the parallel lines on the alignment block.

2.6.4 Chuck modifications

If you are unfortunate enough to end up with wedge shaped otolith blocks, you will find that the normal chuck will not accommodate them properly. The bar will want to sit on too much of an angle and the bolts will not allow the bar to accommodate this angle. A remedy is to slot the holes in the bar with a drill or a file to give the bar more freedom of movement (Figure 12).

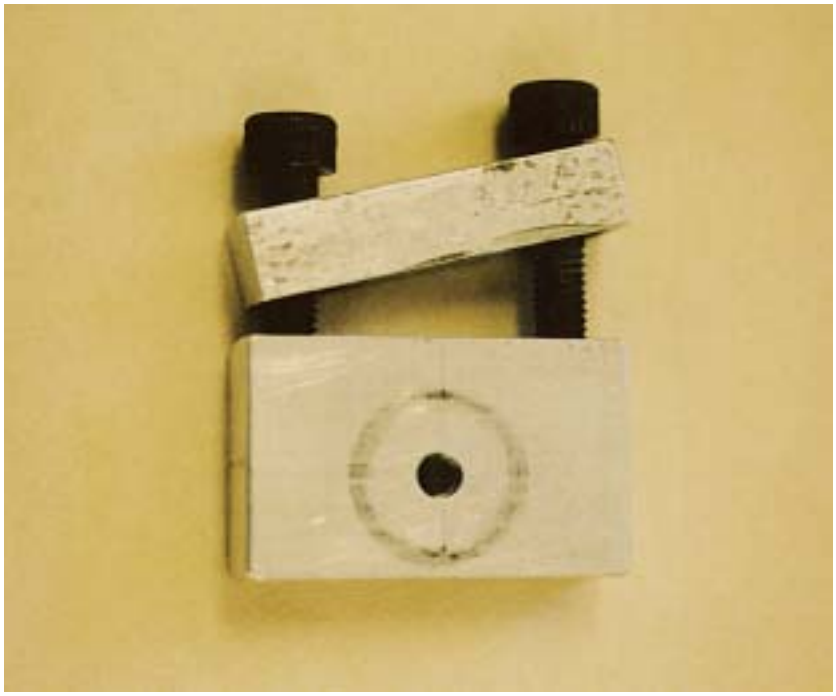


Figure 12. Modified chuck.

2.6.5 Mounting the chuck onto the saw

Attach the chuck to the support arm of the saw with the thumb screw supplied. The sample must be in the lower position (**sulcus upwards**). When tightening up the chuck, move the support bar down slightly so as not to put pressure against the support bar's stops when tightening. When the cutting blade exits the otolith block, it leaves a slight burr along the cut off section. To reduce the length of this burr, the chuck should be tightened up at a slight angle to the support bar (Figure 13) so that the blade exits at the corner of the sample.

Figure 13. Cutting in process, showing chuck and otolith block angle in relation to the cutting blade.

2.6.6 Lining up the otolith block with the saw blade and cutting

The object is to line up the centre of the marked core with the centre of the blade. After checking that the blade is stationary, lower the arm so that the otolith block is just above the blade. Lower your point of view and turn the micrometer adjustment until the top of the blade bisects the dot marking the core. Return the arm to its resting position. Back off the micrometer adjustment to the required amount (see Saw settings), turn on the power and lower the arm. A slower cutting speed produces better sections. A setting of 5 – 6 on the saw's speed dial is generally adequate.

Do not hold the arm freehand as you lower it, as this can deflect the arm from its natural cutting place. Let the arm find its natural (non forced) path by supporting it underneath the chuck thumb screw with the index finger while the wrist is resting on top of the saw cowling. Slowly let the block make contact with the blade. Never drop the otolith block onto the blade.

2.6.7 Before cutting commences

In general, follow the manufacturer's instructions. Here are some additional points to be aware of:

- Always test run the blade to observe for running trueness before any cutting commences. This is done dry, without the lubrication medium tray pulled up into place.
- **Blade wobble** can be attributed to a damaged drive shaft or blade, incorrect assembly (the flanges must have the recesses facing the blade for correct blade support) or residue build up (causing high spots) on the blade or flanges prior to re-assembly.
- When cleaning residue or any other material off the blade, do not hold the blade in the palm of your hand to clean. Make sure that the blade is fully supported on a flat surface.
- Lapping film or the finest available wet and dry paper make a good cleaning medium with a light rub under running water.
- The blade is easier to clean and stays cleaner longer if you periodically spray it with a silicon lubricant.
- It is recommended that you cut three sections from the otolith, with the second slice incorporating the core. Multiple sections can assist greatly with age determination and boost confidence in the results.
- The lubricant pan must be in place before any cutting commences. Use water with a single drop of dishwashing detergent as a lubricant or the manufacturer's lubricant.
- When cutting has stopped for a while (lunch breaks), lower the lubricant pan to prevent a scum line building up on the blade. Swing the pan out to the side as a visual reminder to reposition it after lunch.
- Manufacture a guard of perforated stainless or aluminium sheet, that surrounds the saw blade (Figures 3, 14, Appendix 1) and place it on top of the drip platform. This usually prevents the off cut section from dropping into the lubrication tray.

Figure 14. Entire saw and set up, cutting in progress.

2.7 Saw settings

Calculating the starting point for cutting can be made easy with the creation of a small Excel spreadsheet (Figure 15). The sample here is based on 3 cuts per otolith, with the blade lined up and bisecting the marked core prior to cutting.

Microsoft Excel - OTOLITH Cutting calculations.xls

	A	B	C	D
1				
2	**Buehler ISOMET Low Speed Saw**			
3				
4	**MICROMETER adjustments for Otolith cutting (3 cuts)**			
5	**after the blade has been centred on the Core**			
6				
7				
8	**Blade thickness**	(Micron)	**386**	(supply)
9	**Required cut thickness**	(Micron)	**250**	(supply)
10				
11	**Micrometer graduations back from centre**		**95**	(answer)
12				
13	**Micrometer graduations forward for each**		**64**	(answer)
14	**cut**			
15				
16	**Note:** one turn (360 degrees) of the micrometer = 500 micron			
17	or 50.0 graduations on the micrometer			

Figure 15. Table of cutting calculations.

2.7.1 Spreadsheet formulas

C 11 =SUM ((0.5*C8)+(0.5*C9)+C8+C9)/10
C 13 =C11/1.5

C 11 and C13 have their decimal places set to 0.

As previously mentioned, the **ideal cutting thickness** is a balance between the otolith slice being too thick and opaque to see through and being too thin and translucent to give good observable density to the annuli. For some species, and for microincrements in juveniles, it might be necessary to cut as thin as 120 micron. However, there can be a problem trying to achieve very thin sections (less than 150 micron) because the blade may have a tendency to skip out the side of the cut and then drop down. This action may cause the blade to warp and affect section thickness. For instance, we have had variations of up to 45% of the target thickness of 270 micron. This variation should be measured for your saw and allowed for so that the target core may be more reliably attained, especially if very thin sections are required. If you find that the blade is cutting wider than its measured thickness or if there is a slight blade wobble, there will be an increase in the width of the waste material cut by the blade. This extra wide cut will put you off target for the core but can be allowed for by correspondingly increasing the blade thickness in the spreadsheet formula. To achieve this correction, measure the thickness of several cut sections, being careful to avoid the burr with the verniers (use digital verniers if available). Using the mean of these measured sections as a guide, increase the blade thickness in C8 (blade thickness) in the spreadsheet, until C9 (required cut thickness) corresponds to the mean of your measured sections, then use the micrometer graduation readings as your adjustments (C11 and C13) for cutting. This procedure will give you greater accuracy to the core and cutting thickness predictability, however the cause of blade wobble should ideally be identified and fixed.

2.8 Post-sectioning

2.8.1 HCl

After a section has been cut, dip it into a 2% solution of HCl for up to 15 to 20 seconds and move it around gently using forceps, followed up by a gentle first and second rinse in water (two different containers). This will help to clear the otolith of the embedded calcium carbonate dust on the cut surfaces.

2.8.2 Identifying cut sections

Dry the cut section with low lint tissues or similar, and identify each cut section after drying by writing the identifier number with a pencil, on the resin, alongside the otolith. Using a fine pair of scissors, carefully trim the burr off the cut section and also trim off any large excessive areas of resin to make the mounting onto slides an easier task. Place the sections in groups of three on a flat medium that can be carried away to mount onto slides.

2.8.3 Mounting sections onto slides

To mount otolith sections onto slides, mix only a small amount of **casting resin** at one time. One segment of the ice block tray mould is usually enough for at least 15 slides. Use disposable pipettes (3 ml) to run a narrow strip of resin onto the slide. This has to be broad

enough so that when the cut otolith section is laid across it and pushed down towards the glass, there is enough resin to at least wet the down side of the otolith section with a small amount oozing out around the edges. Leave at least 5 mm of the end of the slide clear of resin or you will have difficulty in accommodating the slide in the slide box. Add just enough resin on top of the sections to cover them and use the pipette to push the cut sections down and into line. Apply the cover slip by touching the end of the slip down onto the slide just beyond the resin and drag it up the slide, against the resin, until the resin spreads a little (dams up) in front of the slip then let it fall parallel onto the slide. If necessary, straighten the cover slip with a pop stick so that it does not overhang the sides of the slide. Check that all otolith sections are covered. Keep the slides in a horizontal position until the resin sets. When dry, view the identification number written on the cut sections and transfer this to the frosted section of the glass.

3.0 Conclusions

The aim of all otolith cutting should be to obtain the best possible readability of any section cut. Rigorous attention to detail as outlined in this article will lead to better and consistent outcomes and will enhance the precision of age determination.

4.0 Do's and Don'ts

Do's

- Do small trial batches to establish cutting thickness.
- Use release agent in the moulds.
- Mix resins thoroughly.
- Only let resin cure whilst it is on an even and horizontal surface.
- Let the bases harden fully before proceeding further.
- Check back after pouring bases to ensure that they are sufficiently covered.
- Cut back large otoliths to shorter length.
- Check that the sample identification is not obscured by the otolith.
- Cut three sections from the otolith.
- Add one drop of dishwashing detergent to the water as lubricant or use manufacturer's lubricant.
- Check that the lubrication pan is in place before cutting.
- Swing the lubrication pan out to the side when not in use.
- Check that the blade is running true before immersing in the water bath or cutting.
- Mark the core accurately.
- Re-check block alignment after tightening into chuck.
- Seat the otolith block far enough into the chuck.
- Check that the clamping bolt's heads are clear of the bar before tightening.
- Support the blade on a flat surface when cleaning it.
- Enhance small otoliths with a pencil mark along the sulcus.
- Display the last identification number used in a prominent place.

Don'ts

- Do not use the anterior and posterior points of the otolith for lining up.
- Do not use warm or hot water in the lubricant pan – you will buckle the blade.
- Do not ever tighten a block in the chuck while the chuck is on the support arm.
- Do not use too much release agent.
- Do not use white moulds for embedding otoliths.
- Do not aerate resin when mixing.
- Do not hold the otolith block up to the light to mark the core.
- Do not lower the arm freehand.
- Do not ever drop the arm onto the blade.
- Do not hold the saw blade in the hand when cleaning it.

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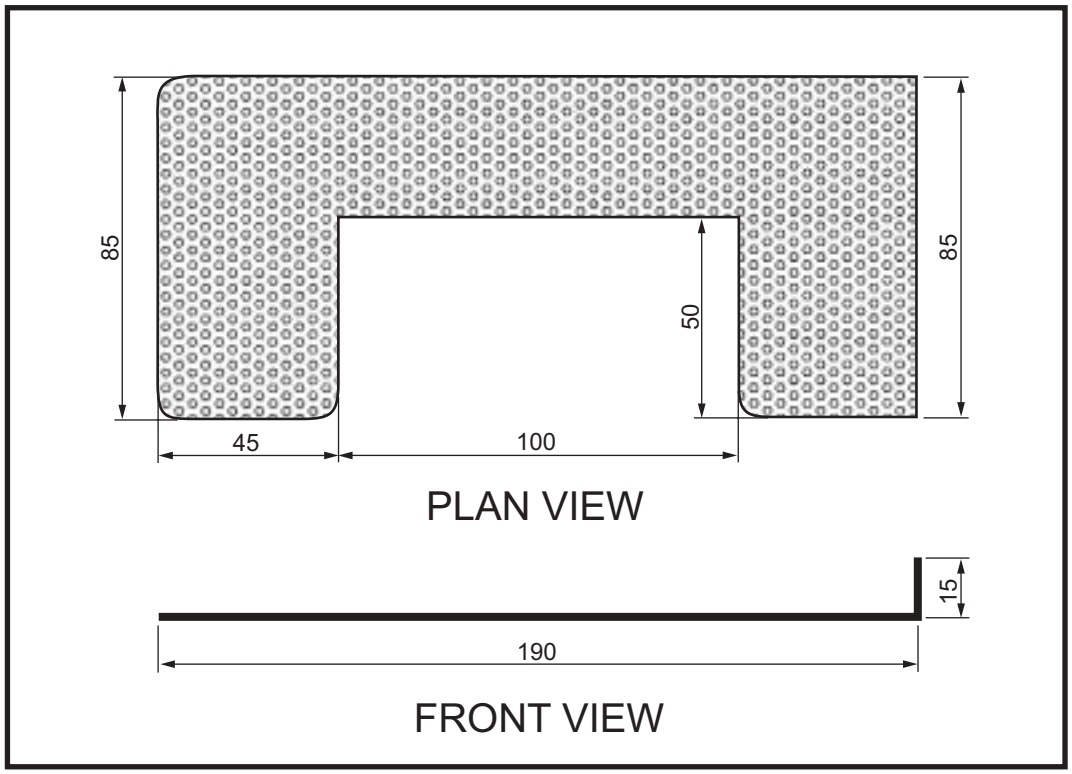
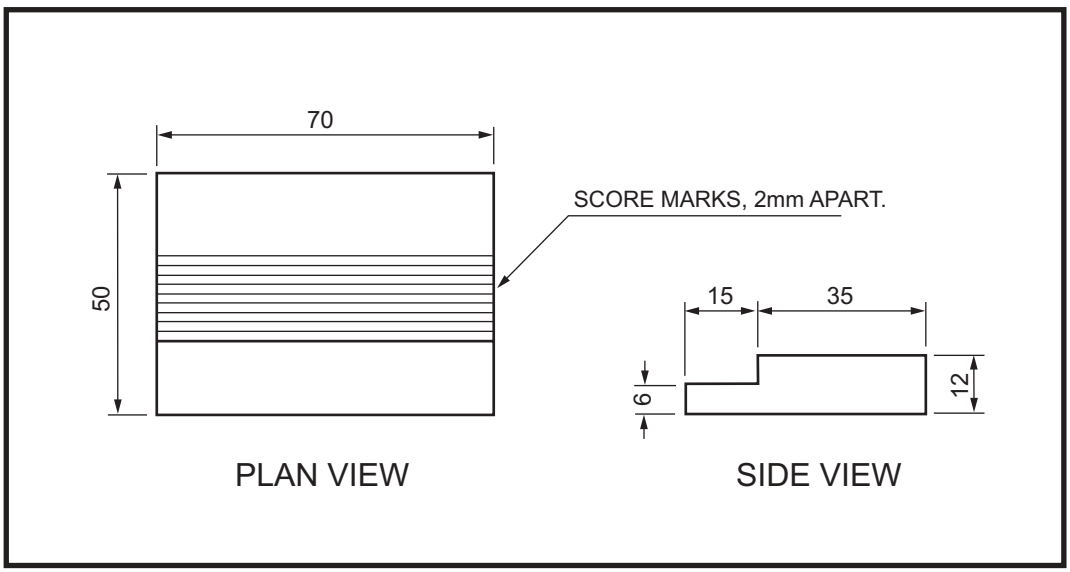
7.0 Appendices

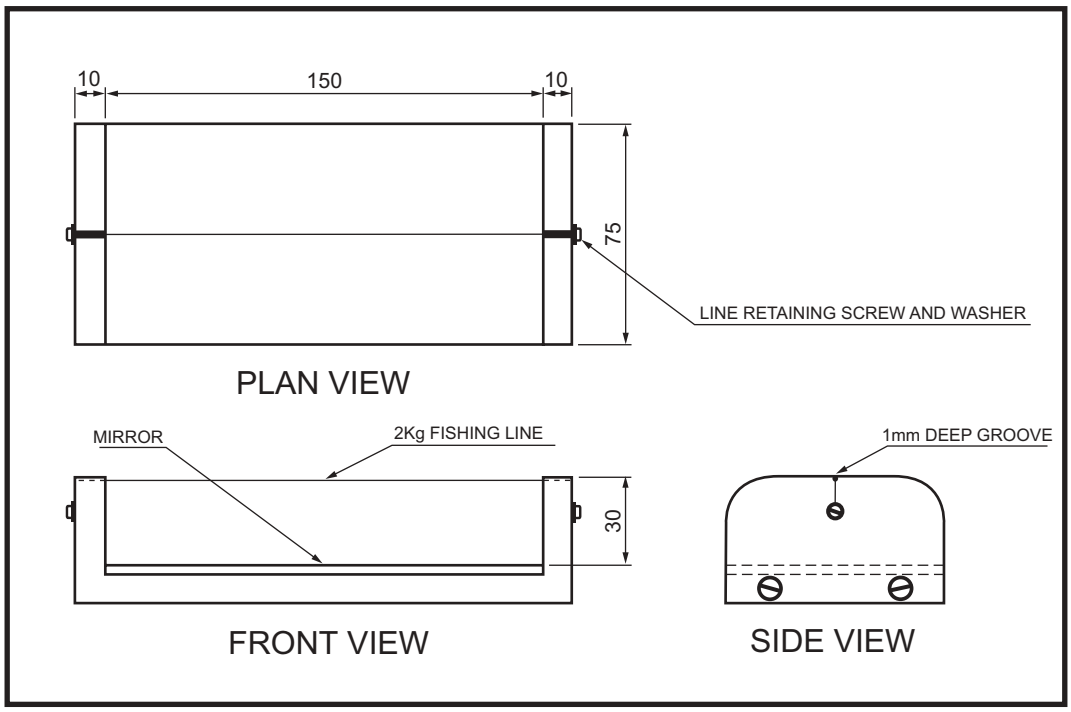
Appendix 1. Design of the perforated guard.



Appendix 2. Design of the alignment block.



Appendix 3. Design of the alignment jig.



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