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Marine Scotland Science

Fish Age Determination Procedures: Gadoids

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FISH AGE DETERMINATION PROCEDURES
1: GADOIDS

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This manual is intended to be used in conjunction with training at the microscope

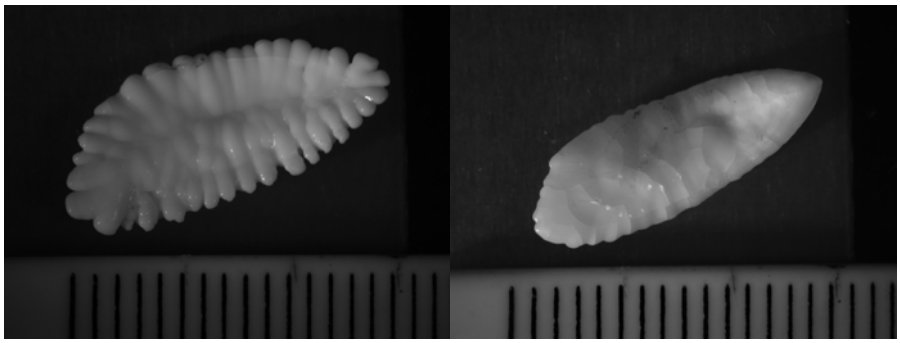
In order to undertake stock assessments for use in fisheries management, a knowledge of fish age/stage is required. Although there is a general relationship in most species between age and size, It is difficult to determine the age of a species by simple measurements of body length or weight. Fisheries scientists, therefore, are required to determine fish age using alternate measures. The most common method is observing the growth rings in hard, bony structures of the fish the ear bones (otolith), scales, spines etc .

This manual describes the procedures employed by Marine Scotland Science for the aging of the Gadoid fish Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*), Whiting (*Merlangius merlangus*) and Saithe (*Pollachius virens*).

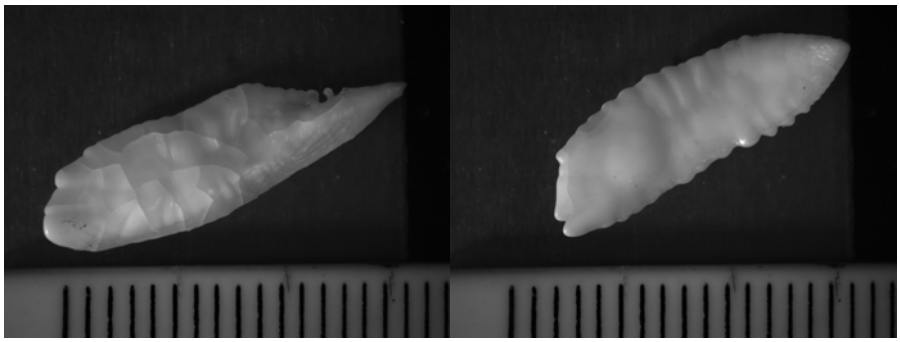
Collection of gadoid otoliths

Prior to collection of the otoliths, the fish are measured on a bespoke scale or measuring board. At fish markets or fish processors this is a wooden board, inlaid with either a 1 cm increment plastic scale for demersal fish, or ½ cm increment scale for pelagic species. On commercial vessels, an aluminium board faced with plastic laminate in a scale increment appropriate to species expected, and which data can be written on, is used. Research vessels have ½ cm measuring scales inlaid in to the work benches. An electronic recording system is used aboard the current research vessel SCOTIA.

Otoliths are removed by one of two methods. Either by lifting the gill covers to expose the auditory capsule then making a single cut with a sharp, stiff bladed, knife into the auditory capsule and exposing the otolith, or by making a transverse cut across the head of the fish to open the auditory capsule. In either case, the otolith(s) can be removed with the knife or forceps.



Cod otolith in May Haddock otolith in May



Whiting otolith in May Saithe otolith in May.

Figure 1. Otoliths of the four main gadoid species. Scale in millimetres.

One otolith from each pair is placed into a single paper packet with the fish length recorded on the front. Full details of the sample are recorded on a header packet and on the associated length recording sheet, according to the Market Sampling Instructions and Codes.

Preparing gadoid otoliths

Otoliths (fig 1) of cod, haddock, whiting, and saithe are prepared for analysis in the field by the staff collecting them. The otoliths collected by North Atlantic Fisheries College (NAFC) in the Shetland islands are sent to Aberdeen and processed in the laboratory. Norway pout (*Trisopterus esmarkii)* otoliths are processed at sea.

Otoliths are removed from their packets by inserting a finger or a blunt object into the packet and carefully dislodging the otolith. Care should be taken to ensure fragile otoliths are not damaged at this point, particularly the small and delicate otoliths of Norway pout. They are placed in sequential order on a vinyl cutting mat, with their distal surfaces uppermost, ready for "breaking" with a scalpel (fig 2).

Figure 2. otolith ready for breaking

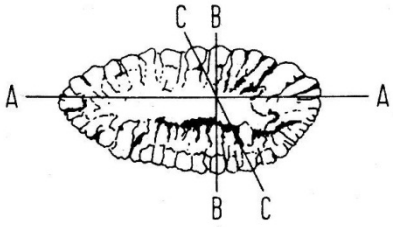
A suitable mount (fig 3), in this case a jar top filled with black plasticene, has to be prepared to receive the otoliths. This is done by smoothing the surface of the plasticene with the thumb or with a scalpel handle or similar flat object. It is very important that the resultant surface is level with the top of the mount. If the plasticene builds up behind the otoliths when they are mounted, light will be reflected back through the otolith instead of passing upwards through it, making interpretation difficult. A similar effect is observed when the otoliths are mounted too deeply in to the surface of the plasticene. Care is needed to avoided mounting the otoliths too deep (fig 4). Otoliths should be mounted on the rim to a depth of allowing for approximately 5mm above the mount.

Figure 3. Mount with plasticene build up behind the otoliths.

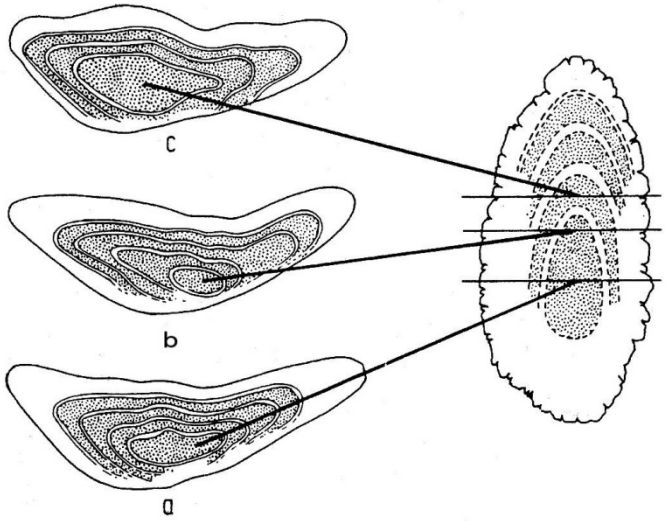
Figure 4. Mount showing otoliths too deeply embedded i.e. only 2mm showing

It is standard practice to score the otolith on its distal surface with a scalpel blade. The exact position of the score mark is very important, as the aim is to break the otolith through the nucleus (fig 5). Failure to do so can cause problems for the reader, as missing the nucleus can alter the apparent structure of the otolith rings and cause difficulties in interpretation, such as underestimating the age by one, or more, years.

The section through B to B, in Figure 5 below, is most commonly used in MSS.



Three possible sections through a cod otolith:
A-A longitudinal; B-B lateral; C-C diagonal.



The varying appearance of the ring structure of a cod otolith when broken.

(a) through the centre of the nucleus
(b) through one end of the nucleus
(c) missing the nucleus completely

Figure 5. Diagram showing the importance of an accurate cut on the otolith.

The position of the nucleus can be derived from the presence of a small raised bump on the distal surface, or a small pit on the sulcus acusticus on the proximal surface.

Having identified the nucleus, a series of light scoring motions are made across the nucleus and transversally to the anterior - posterior axis. Light finger pressure applied at either end of the otolith should then result in a clean break (Fig 6.). This technique should be closely followed to prevent fragile otoliths, (of whiting in particular), from shattering.

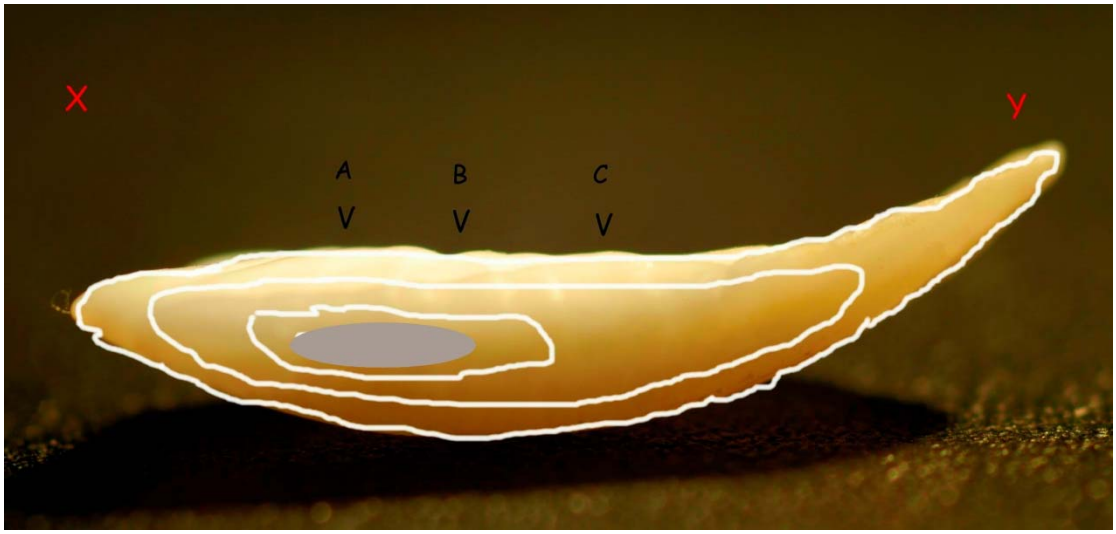


Figure 6. Otolith should be scored at position B. Finger pressure applied at X and Y will ensure break happens at the correct location to show all growth zones.

The mount is marked with an “L” shape, and then the otoliths are mounted in a clockwise direction from this point. The posterior end of the otolith is mounted vertically, with the cut side uppermost and the proximal surface facing outward. This is usually achieved by holding the broken otolith between the thumb and forefinger although, for the very small otoliths of Norway pout, it may be necessary to use forceps.

Figure 7. Top view of mounting in groups of five with a gap

Figure 8. Side-view mounting in groups of five with a gap.

Otoliths are mounted around the circumference of the mount in groups of five, with a small gap separating each group (Fig 7 and Fig 8). Every fifth packet is marked with a pencil line on the top right hand corner to correspond to the groups of otoliths (Fig 9).

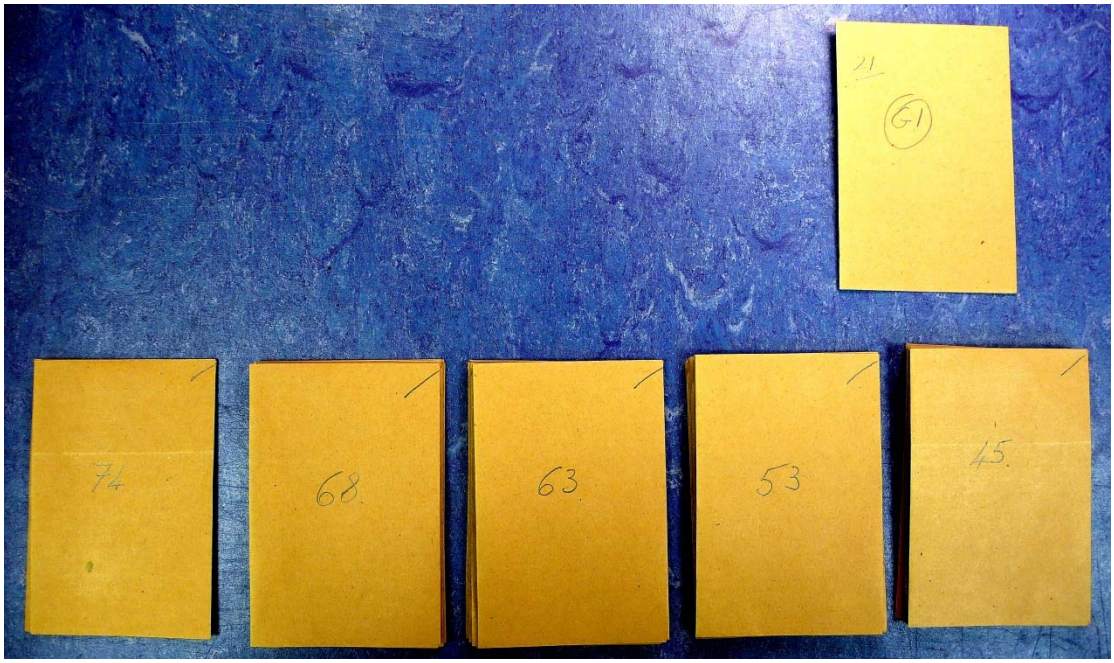


Figure 9. Packeted groups of five otoliths marked with a pencil and size catergory

The packets are retained and kept in sequential order to correlate to the mounts, secured with an elastic band and stored in a box with the completed mounts. The number of otoliths in each size category is recorded on the top left hand corner of the header packet of each size category.

Brittle otoliths of a glassy appearance may be encountered during preparation for reading. The otoliths are variously described as “decalcified”, “crystal” or “calcified”, but the correct term is vateritic (Fig 10). These otoliths are generally unusable for age determination purposes, and should be discarded. The phrase “**NO OTO**” should be written on the corresponding packet so that the reader is aware that an age is not available at that length, and does not inadvertently record an age on the wrong envelope whilst reading the sample.

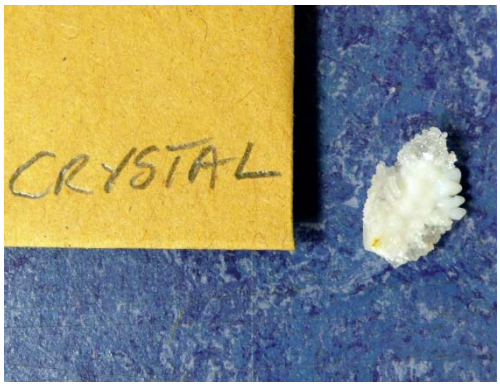


Figure 10. Picture showing vateritic otolith unsuitable for age reading purposes.

INTERPRETATION TECHNIQUES

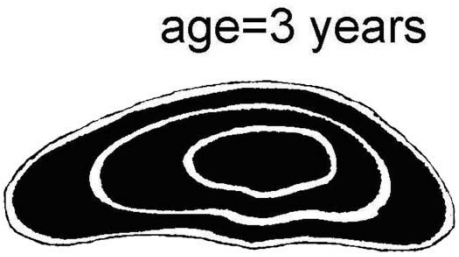
To view an otolith a number of techniques are available. The simplest of these is to immerse the whole otolith in a clear liquid (water is quite commonly used), illuminate it from above, and view it against a dark background. This method is suitable only if the otoliths are relatively thin and translucent and all the rings can be seen (e.g., anglerfish, megrim, herring and mackerel).

In many species the outer rings become very narrow once the growth rate of the fish slows down. These narrow rings sometimes grow only on the underside of the otolith, and are completely invisible when the whole otolith is viewed from above in the manner described. They can be seen only when a cross section of the otolith is viewed. When investigating any species of fish it is always necessary to check, by examining a cross section, whether these narrow rings are present before accepting an age based on viewing the whole otolith from above. Failure to understand this type of growth pattern in otoliths can result in gross underestimates of age.

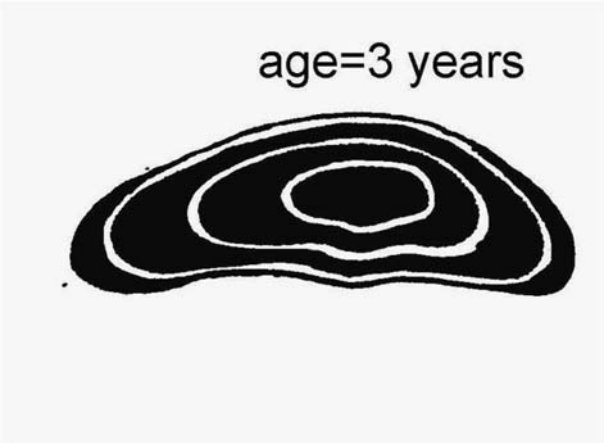
The age of a fish at a given time refers to the period of time from birth to that given point of time. In order to age a particular fish, it is essential that the date of capture of the fish is known. When the age of a particular fish has been determined, it is assigned to the appropriate age group which is an integral number of years according to a convention based on an arbitrarily adopted birthday. **There is an internationally accepted convention to use 1st January for most of the North Atlantic demersal fish species. This means that there can be no ambiguity about the year class to which a fish belongs and all data are comparable.**

For example, a fish spawned in May 1996 belongs to the 1996 year class and is assigned to age group 0 until 31st December 1996. On 1st January 1997 the fish is assigned to age group 1 when it is 9 months old. On 1st January 1998 the fish belongs to age group 2 when it is 21 months old. If this fish was captured in September 1998 it would be 29 months old and be assigned to age group 2.

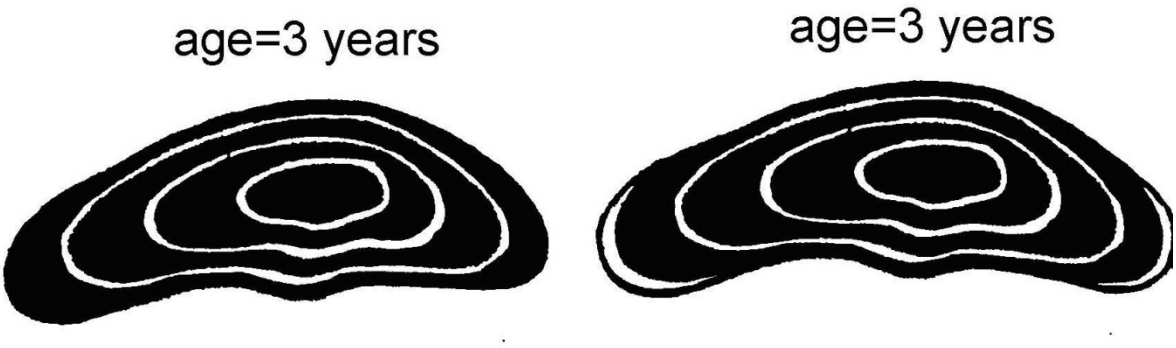
Although somewhat exaggerated, the drawings below adapted from Norwegian literature (Anon., 2000 –ref 1) demonstrate the principle for gadoids (Figure 11):



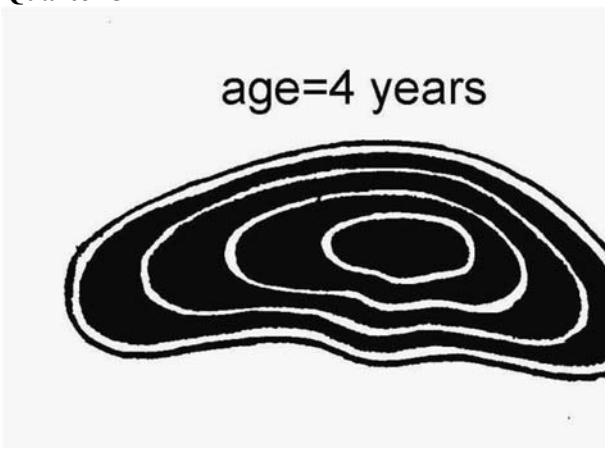
Quarter 1



Quarter 2



Quarter 3 **Quarter 4**



Quarter 1

Figure 11. Drawings to show the development, over five seasons, of a new otolith growth increment

To carry out the tasks listed in this procedure in the best possible way, it is vital that the persons involved are acquainted with the protocol described below. Also, it is vital that they maintain their knowledge and skill by processing a reasonable amount of otoliths each year.

It is important that the work area is organised in such a way that the otolith reader can look easily into the microscope without too much tension, and that the microscope is focused correctly. Interpretation of otoliths is demanding work and should not be carried out for long periods without pause or change of work.

The first step is to prepare the microscope for use. Make sure that the eyepieces are clean and that the microscope and chair are adjusted for operator comfort, as it is likely that an analyst may spend several hours processing samples at a time. Although optimal magnification rates vary by species, for gadoids the range of x10 to x15 is deemed most useful, and is selected according to individual reader preference.

Operating a microscope for long hours will strain the neck, shoulders, eyes, lower back, arms and wrists. Working at a microscope that is not at the correct height and angle, requires a hunched position and causes contact stress on the forearms from the work surface edge. Avoid long uninterrupted periods of microscope work by rotating tasks and taking frequent rest breaks. Every 15 minutes, close your eyes or focus on something distant. Every 30-60 minutes, get up to stretch and move. **This is most important for preventing injuries, as well as improving how you feel at the end of the work day.**

Otoliths are read in a clockwise direction around the mount starting at the “L” shaped indicator which ensures that they match the order of the packets.

Prior to examination under the microscope, all otoliths on the mounts are brushed with mineral oil (baby oil) using a small paint brush. This cleans the surface of the otolith and provides a refractive index which aids interpretation of the annual rings.

Figure 12. Photograph showing equipment setup used for age reading of gadoid otoliths (cod, haddock, whiting and saithe).

The prepared mount is placed on the microscope stage and the light source adjusted to focus the beam onto the proximal surface of the otoliths (Fig 12). When viewing the otoliths through the microscope, the contrast between opaque and translucent zones will be improved by interrupting the light beam with the index finger of the left hand, thus making the zones clearer and more distinct. The opaque zones should be counted from the centre to the outer edge and care must be taken to follow the zones around the distal and proximal sides to check for splitting or merging of the zones before estimating an age (Fig 13 and 14). In older fish the zones should be counted at several places on the otolith surface to ascertain a consistent figure. Should an otolith, particularly of older cod and saithe present problems with interpretation, it may be useful to grind the cut surface on a wet stone grinder as illustrated (Figure 15).

When an age has been derived for the otolith, the age is recorded using a pencil on the bottom right hand corner of the corresponding packet.

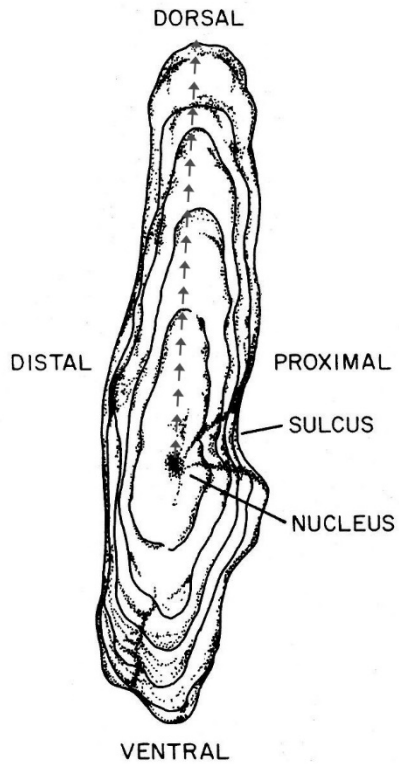


Figure 13. Image of broken otolith with principal features and preferred reading axis (arrowed).

Figure 14. Image of an otolith from a four year – old haddock with growth (opaque) zones marked in red. Fish captured in Q1 2009, as viewed under microscope.

Figure 15. A wet stone grinder as used to grind flat the cut surface of otoliths which have not broken cleanly.

The following features apply to gadoids in general:
(Excerpt from) 5CA-2002-01891
TOWARDS ACCREDITATION AND CERTIFICATION OF AGE
DETERMINATION OF AQUATIC RESOURCES
(TACADAR)
FINAL REPORT
For the period
1 October 2002 to 31 October 2006

Types of notable features found in Cod otoliths

•juvenile zone: close to the nucleus is often a small, clear and even ring. This is not counted when assigning age

• translucent zones: in general are regular and concentric but their translucency may vary. Generally they have the same shape as the otolith section. They can sometimes split in certain otolith areas. Also a group of multiple rings, forming a zone, are found and are identified based on their relative spacing

• false translucent zones: in general a thin often diffuse ring that is not visible around the whole otolith and does not follow the overall pattern of the annual increments of the otolith

• first translucent zone: distinct, regular and concentric not to be confused with the smaller juvenile zone

• problem otoliths: the first translucent increment is often a double ring especially for fish up to two years. In certain stocks and in older fish the formation of the translucent zone can occur very late in the year and sometimes the reader has to assume the formation of this increment in the edge

Saithe and haddock are regarded as relatively easy to age and present few problems. Older saithe tend to be the source of most differences between trainer and trainee as the growth rings become very tightly packed on the otolith edge. However, some of the features described in cod otoliths may also be found in haddock and saithe.

Problems associated with the age estimation of whiting

Whiting is considered one of the more difficult species to age due to the presence in some otoliths of unusual growth features such as double zones, Bowers zone and Humphries shadow.

Double zones, or splits, are formed from two thin translucent zones separated by an opaque zone, and can usually be resolved by following the zones around the otolith to where they unite to form a single true zone.

Bowers zone: this is a single false zone where the nucleus is bounded by a thin translucent zone inside the first true winter zone, (Gambell and Messtorff, 1964).

Humphries shadow: this is an opaque area lying within the annual translucent increment on the internal face of the rostrum (mainly on dorsal area) (Anon., 1987).

Anon. 2000. Procedure for age estimation of cod, haddock and saithe". Version 1.0 Institute of Marine Research, December 2000, Hildegunn Mjanger, Kjell Nedreaas, Haral Senneset and Per Agotnes. Translated in to English by Bente Lundin, Institute of Marine research,

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