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Yellowtail Flounder *(Limanda ferruginea)* Ageing Manual

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Abstract

This is a technical manual describing the methods and interpretations used for estimating age in yellowtail flounder (*Limanda ferruginea*). The paper gives a general overview of ageing, and then discusses how yellowtail flounder are presently being aged at the Northwest Atlantic Fisheries Centre (NAFC) in St. John's, Newfoundland. It also provides information on the types of validation studies that are used to ensure accuracy of ageing, and attempts to troubleshoot any difficult aspects of ageing. The thin-sectioning method used to age this species is discussed, and includes detailed information on how it is carried out. The structure of the whole otolith were discussed, along with the limitations for ageing yellowtail flounder using this structure. This manual contains a glossary and high quality photos and diagrams for use when ageing yellowtail flounder.

Introduction

Age of marine fish can be determined by counting periodic markings on a variety of 'hardparts'. Scales, vertebrae, spines and otoliths have been used to determine age in fish. In the case of flatfish otoliths are unique in that they have both symmetrical and asymmetrical sagittal otolith (Hunt, 1992).

Age of fish is essential in fisheries management. Hence, accurate age determinations are a vital part of the scientific process of stock assessment and are critical for estimating mortality and growth rates (Chilton and Beamish, 1982; Penttila and Dery, 1988). Otolith growth is related to somatic growth; as the fish grows so does the otolith. While somatic growth periods (of soft tissue) is not discerable, the growth of the otoliths deposit discernable patterns where a year's growth consists of one opaque zone and one translucent zone, which compose an annual zone. Alternating zones on the otolith are due to differences in protein deposition and the shape of aragonite crystals present in the otolith (Degens *et al.*, 1969; Penttila and Dery, 1988). The opaque zone is formed during the period of faster growth, usually in the summer/autumn and the translucent zone is formed during the period of slow growth, usually in the winter. Sometimes the zones are referred to as summer and winter zones, respectively.

The terms opaque and translucent are relative terms, because they refer to how the summer and winter zones appear when otolith sections are illuminated. Opaque zones appear white under reflected light and the translucent zones appear dark but the opposite is the case when the otolith is examined under transmitted light. Yellowtail flounder are usually examined under reflected light, so the terms "opaque" and "translucent" refer to white and dark zones, respectively. In this manual, when opaque or translucent zones are discussed, they refer to those as seen under reflected light.

Younger fish display otoliths with wide opaque zones because otolith growth is rapid. As the fish grows older, the width of the annulus decreases. Eventually, the opaque zones become more and more narrow until they are the same width as the translucent zones.

Yellowtail flounder, *Limanda ferruginea*, is a small-mouthed right-eyed pleuronectid found in the Northwest Atlantic. It is distributed from northern Newfoundland south to Chesapeake Bay, including the Gulf of St. Lawrence (Scott and Scott, 1988). Yellowtail flounder are fished commercially on the Grand Bank off Newfoundland, as well as south on the Scotian Shelf, Georges Bank and off Cape Cod.

Flatfish at the Northwest Atlantic Fisheries Centre (NAFC) are aged mostly (some species' otoliths are broken) by reading the surface of whole otoliths and counting the dark zones, or annuli. By tradition, yellowtail flounder were aged using scales and whole otoliths (Pitt 1974; Royce *et al*., 1959; Scott, 1947). At the time of writing, southern stocks of yellowtail flounder are still aged using scales. This is more appropriate for these stocks, in which fish are fast-growing and do not commonly reach as high a maximum age as other flounder stocks (about 7 years old (Penttila and Dery, 1988)).

Accuracy refers to how close the estimated age is to the true age of the fish and it is determined by validating age methodology. At NAFC, both the whole and thin-sectioned otolith techniques have been validated using a number of techniques. Methods include length-frequency analysis, measuring the first annulus, examining otoliths from cultured fish, as well as marginal increment, tag-recapture and bomb radiocarbon analyses (Dwyer *et al*., submitted). Whole otoliths appear to be accurate for ageing yellowtail flounder up to 7 years (between 30–35 cm), although it is considered best at present to only use this method for fish up to 25 cm. Thin-sections are the most accurate method for ageing older yellowtail flounder, although even with this method it is difficult to distinguish between annuli at the very oldest ages. Thin-sectioned otoliths have been validated for a number of species, such as redfish (*Sebastes mentella*) (Campana *et al*., 1990), Pacific grenadier (*Coryphaenoides acrolepis*) (Andrews *et al*., 1999), black drum (*Macruronus novaezelandiae*) (Kalish *et al*., 1997), haddock (*Melanogrammus aeglefinus*) (Campana, 1997) and southern bluefish tuna (*Thunnus maccoyii*) (Kalish *et al*., 1996).

Sagittal otoliths are collected at sea via a length- and sex-stratified random sample (usually 1 cm/sex/set/strata) (details can be found in Doubleday, MS 1981) and have been collected from research vessels since 1949 and commercial vessels since 1969. Both otoliths are collected and stored dry in an envelope. When sectioning is required, each section is placed in a small paper folder and returned to the envelope. This may change when a mass-production method is used to section otoliths but this has not been implemented yet at NAFC. When it is implemented, the otoliths sections will be placed on microscope slides.

A dissecting scope is used to read either whole or sectioned otoliths. Otoliths are observed at 7.5 X to 50 X with reflected light from fibre optic light sources; surface or whole otolith ageing is done using the lower end of magnification (7.5–20 X) and sectioned otoliths read using the higher end (25–40X). Information provided on the envelope is time of capture and the sex of the fish. Length of the fish is also given, although there are differing opinions on whether this may cause bias in age determination. Age is recorded, along with edge type and reliability codes (1 = extremely difficult to interpret annuli to 4 = annuli are clear and easy to interpret). Quality control is being considered at NAFC by creating a reference collection for each species that will be representative of the otoliths in the stock. The reference collection will be read periodically to measure within- and between-reader drift, and can be used for training. To ensure that changes in patterns over time are taken into account, recent otoliths should be added to the reference collection regularly. As well, readers will be tested using age bias plots and coefficient of variation (%) (Campana *et al*., 1995). Precision refers to the closeness of two or more age readings and should be as high as possible (ie. variation should be low). In addition, there will be some attempt at exchanges between labs (ex. St. Andrews Biological Station, National Marine Fisheries Service Northeast Fisheries Science Centre); however this remains a difficulty as labs use different ageing methodology for yellowtail flounder. There was a yellowtail flounder age reading workshop in 2001, which provided an opportunity for discussion of such issues and the implementation of a number of quality control tests (Walsh and Burnett, MS 2001).

As mentioned, like other fish, growth is faster in younger yellowtail flounder compared to older ones. As the fish grows older, otolith growth is deposited laterally, and as a result, some annuli are not visible on the surface of the otolith (see Figures in General Ageing Section). Therefore a cross-section is used to age the fish once it reaches a certain size (~30 cm). This technique is similar to one described in Penttila and Dery (1988), with some minor modifications (see Thin-Sectioning Preparation Section).

Training is done using the reference collection and a senior ager. The senior ager shows the trainee typical growth patterns and allows the trainee to develop an image search pattern, by using a small number of otolith (approximately 100). Afterwards, the senior ager gives the trainee a number of otoliths to age on their own, and these otoliths should be tested against the senior agers' original ages. Bias should be negligible; in general, a rule-of-thumb is that bias not be present for more than ±0.75 years >3 years consecutively and the CV should be between 5–7%. The ager may chose to pick a number of these otoliths for discussion depending on how the testing goes, or if there are a particular number of disagreements. The trainee should be checked against a reference collection frequently once production ageing begins.

Yellowtail flounder have been aged (using the thin-sectioning technique) up to 25 years. There are some problems with this method of ageing yellowtail flounder, including the interpretation of the first years of life, the “transition zone” (the area at which growth slows and switches from “juvenile” to “mature”) and edge growth. Annuli from the oldest fish are difficult to interpret. This manual describes how to prepare and read from both whole and thin-sectioned yellowtail flounder otoliths in order to accurately estimate age.

General Ageing

Age determinations of fish species are carried out using a number of hard parts or structures, including otoliths, scales, bone, vertebra, and spines. Otoliths are structures also called “ear bones” and are most commonly used for ageing marine fish. They are composed of calcium carbonate in the crystalline form of *aragonite*, an inorganic material, along with small amounts of the organic material otolin, in a protein matrix (Dannevig, 1956; Degens *et al*., 1969). Translucent zones, however, are composed primarily of otolin with reduced aragonite, and opaque zones are composed of aragonite needles which are long and thick relative to those in the translucent zones (Degens *et al*., 1969; Penttila and Dery, 1988).

Yellowtail flounder are currently aged at the NAFC by use of whole otoliths up to a length of 25 cm, and thin-sectioning method for all fish >25 cm. Figures 1 and 2 show a section and a whole otolith from the same fish. At sizes less than 25 cm, there are usually no discrepancies in ages estimated between methods. Figure 3, however, demonstrates the situation whereby the whole otolith method does not detect the slower, lateral growth that occurs in older fish.

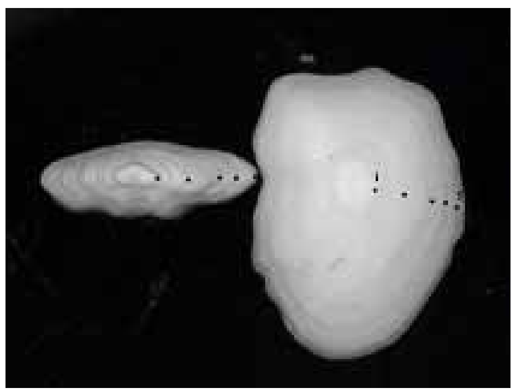
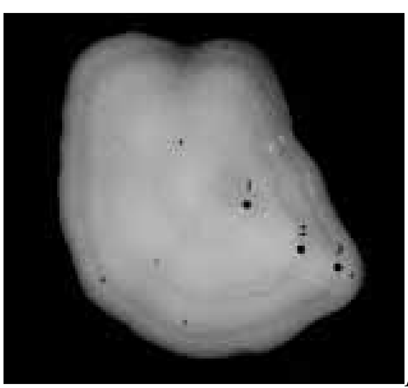


Fig. 1. Section and whole otolith from the same fish compared. It is 5 years old and a 20-cm male yellowtail flounder. Annuli are indicated by dots. Fish was captured in October/November 1998.


A.

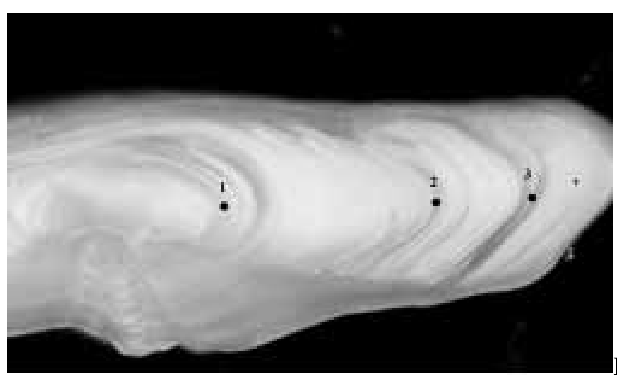
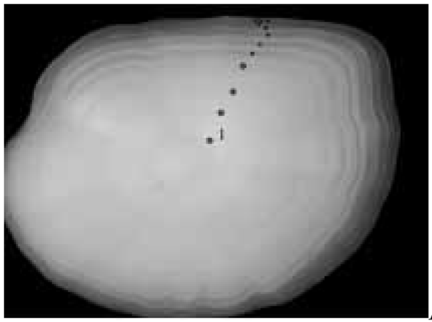
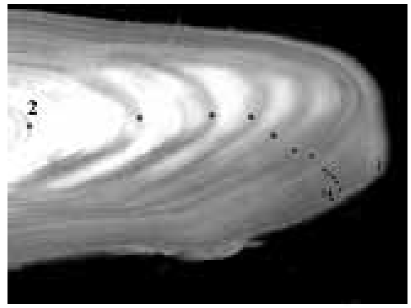

B.

Fig. 2. The same otolith, read whole (A) and sectioned (B). This fish was 23-cm female and aged as 3 years old. It was captured in November 1987. Annuli are indicated by dots and edge growth is also indicated by “+”.


A.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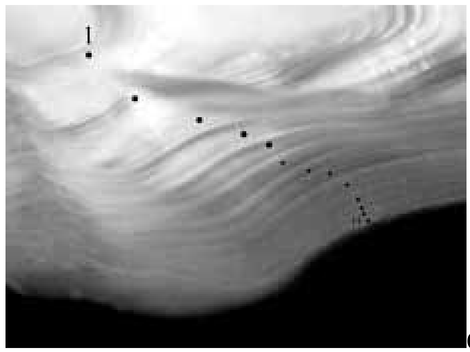

C.

Fig. 3. Comparison between the whole otolith (A) and section (B) from a 42-cm female fish captured October/November 1998. At this size, the whole otolith method fails and more annuli are seen on the section (9 years old as compared to 14 years old). Fig. 3C shows a close-up of the sectioned otolith.

Flatfish have two different shaped otoliths (see Fig. 4 and 5), the left or dorsal otolith being the most symmetrical (nucleus is close to being in the centre of the otolith) and therefore easier to read, whereas the right, or ventral otolith, is asymmetrical and generally more difficult to read (in cross-section is more convex – and sulcus more compressed).

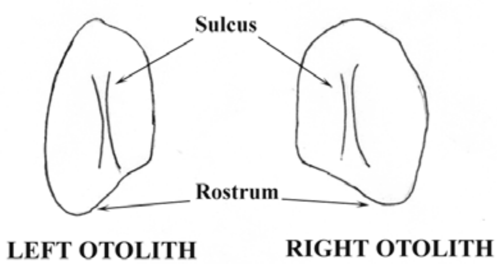
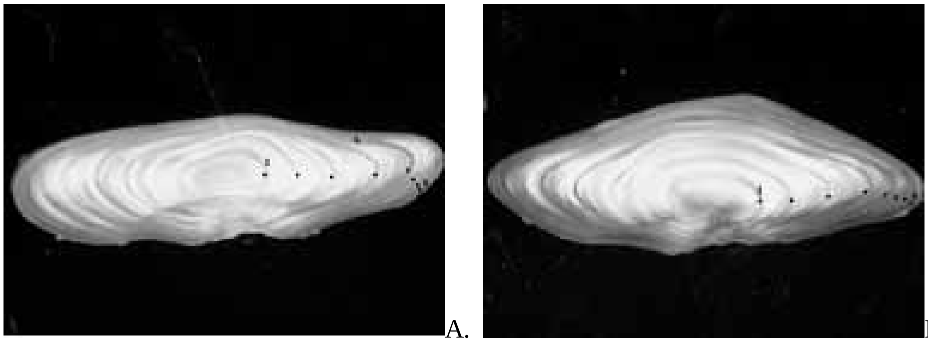


Fig. 4. Drawing of left and right otoliths.

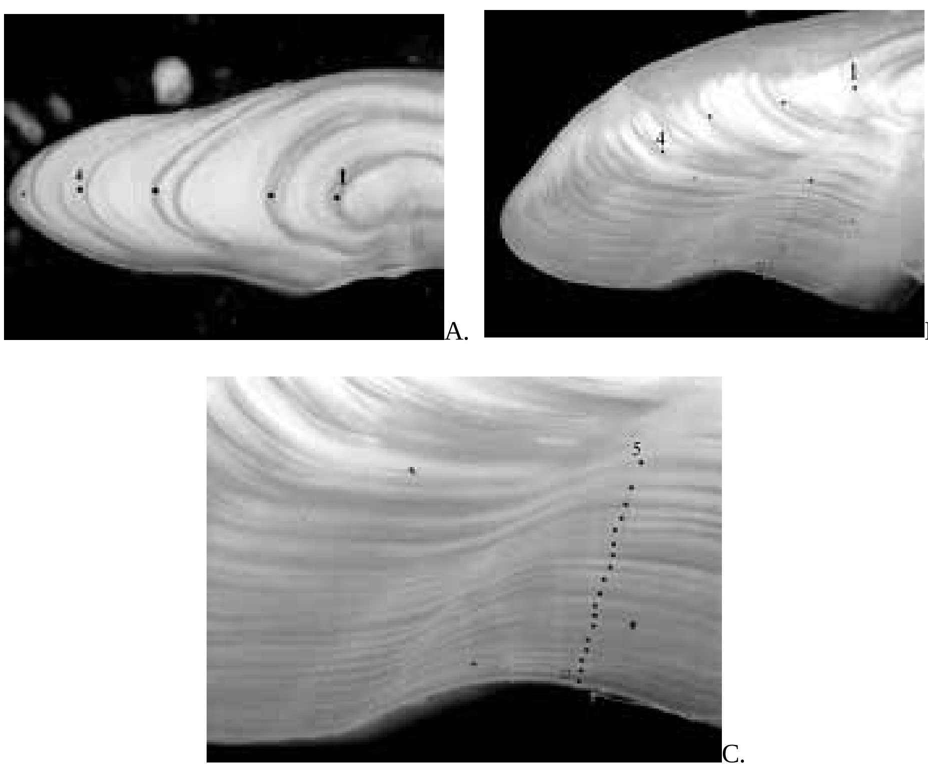


A. B.

Fig. 5. Left (A) and right (B) sectioned otoliths from 34-cm male yellowtail flounder captured in November 2000.

While the two otoliths are thought to have an equal number of annuli, often fewer annuli are counted from the right otolith, possibly due to the fact that the edges are more compressed. For this reason, although both otoliths should be examined, it is standard to use the number of annuli counted from the left otolith at NAFC.

An "annulus" is defined as a continuous translucent zone and is deposited during winter months and is considered to form once per year. In younger fish, the annuli are wider. There are also more checks throughout the opaque zone in younger fish than in older fish. In otoliths of older fish, the winter and summer growth zones are almost equal in size. Figure 6A shows an otolith from a younger fish, with wide opaque zones, whereas Fig. 6B and C show an otolith section for an older fish, which shows much narrower opaque zones.



A. B. C.

Fig. 6. Otoliths showing the difference in patterns in growth between young fish (A) and old fish (B). Figure 6A shows an otolith from 18-cm male, captured in November 2000, and Fig. 6B shows an otolith from 56-cm female, captured in August 1988. Fig. 6C shows higher magnification of the outer annuli.

Axes to Read

Different counting axes are used for different methods. In thin sections, growth along the longest axis tends to be "stretched out" and checks are very prominent. However, because annuli appear very close together in the sulcal area, the best axis to read tends to be along Axis II (Fig. 7) but readers should try to follow annuli back to the sulcus area (Axis III).

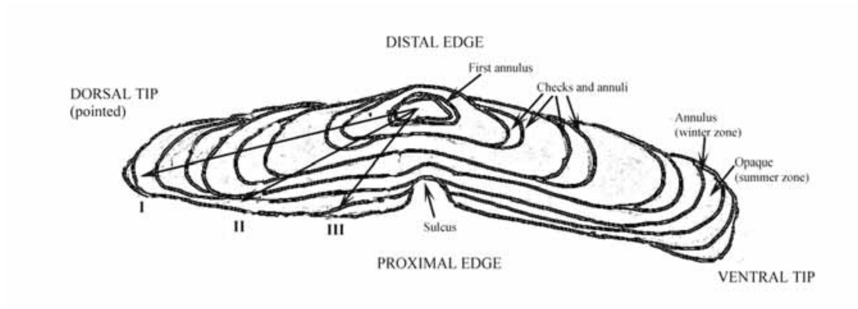


Fig. 7. Drawing of a yellowtail flounder otolith (modified from MacLellan, 1997) section showing areas used for counting and typical ageing characteristics. Axis I indicates fast growth and axes II and III indicate areas of slower growth. Any of the axes can be used to age yellowtail flounder but often using axis I and tracing it into axes II and III is preferred.

It is useful to assess the completeness of annual zones at the sulcus by following the annuli (see Fig. 8).

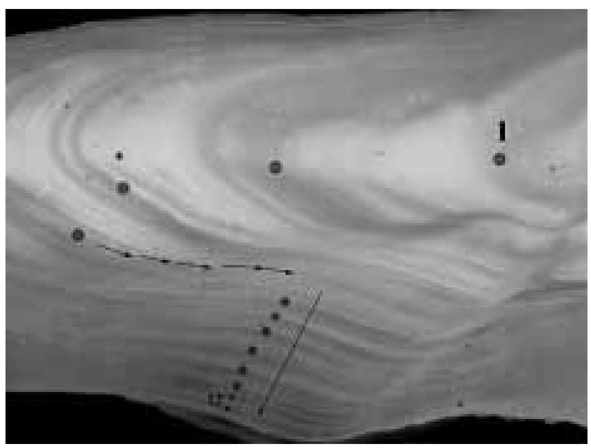


Fig. 8. Annuli can be followed in the sulcus area and are sometimes easier to see there. This flounder is a 12 year-old female and is 52 cm, captured in May 1983.

Age Designation

Yellowtail flounder hatch in spring or summer (Scott and Scott, 1988) but conventionally they are assigned to an age class by using January 1st as a standard birthdate. The number of annuli is counted on the otolith and then must be assigned to an age class based on the time of year captured and the edge type. Using this convention, a fish captured in November with 4 full annuli and 1 forming on the edge would be considered age 4. The same fish, captured in January would be considered age 5. Young flounder, which hatched early in the summer, for example, will be picked up by surveys in the fall and will appear in length-frequency plots then. Therefore, especially in younger fish, when growth is relatively fast, length-at-age will change over the year. Thus, length should not be used to estimate the age of a fish; however, it can sometimes be used to assist the reader after an age has been determined. See Fig. 9 and 10 for more details.

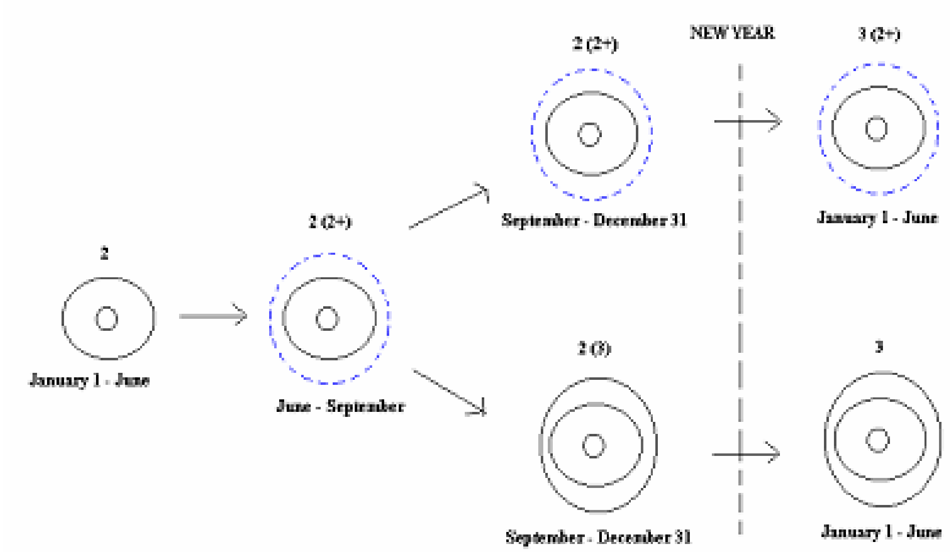


Fig. 9. Ageing according to the January 1st conventional birthdate. These drawings represent an otolith showing growth throughout the year. Solid lines represent annuli and dashed lines represent incomplete summer growth. The numbers above each represent the age that would be assigned the fish based on the January 1st birthdate, while the number in brackets indicates the number of annuli actually seen. Modified from MacLellan, 1997.

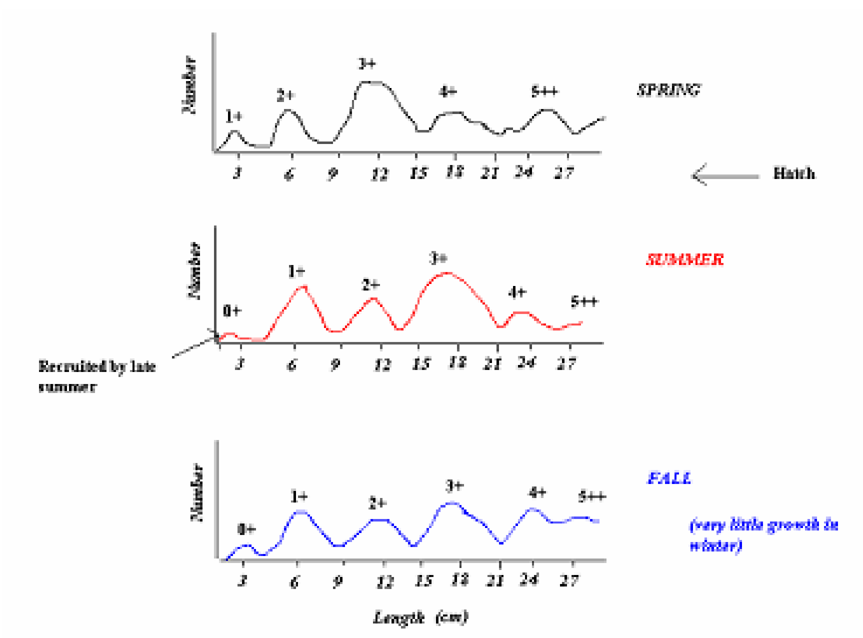


Fig.10. diagram showing a sample length-frequency plot and how modes change throughout the year. Using length-at-age for fish can be misleading because it changes over the year.

First annulus

In yellowtail flounder, the first annular zone is very small, the second to fifth or seventh are large and the subsequent annular zones very narrow. Figure 11 shows a sectioned otolith from a 2-year old yellowtail flounder that shows a typical growth pattern for the first two years. Figure 12 shows sectioned otoliths from fish of different sizes; the main point to note is that the first annulus is very consistent, even when other growth zones vary in size. It is unusual for a previous annual zone to be less in width than the following one, but in some cases it does happen (see Fig. 38, in Reading Thin-Sectioned Otoliths Section). Typically the size of the annual zones begins to diminish after sexual maturity with respect to that of juvenile zones and this is known as a "transition zone".

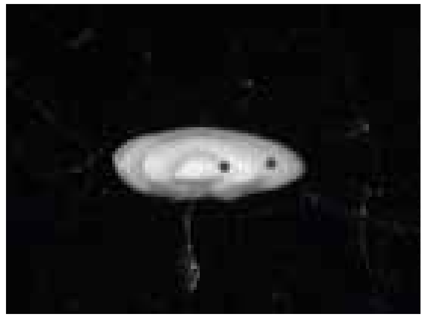


Fig.11. A 2 year-old male 12-cm yellowtail flounder otolith showing typical growth patterns in young fish. It was captured October/November 1999.

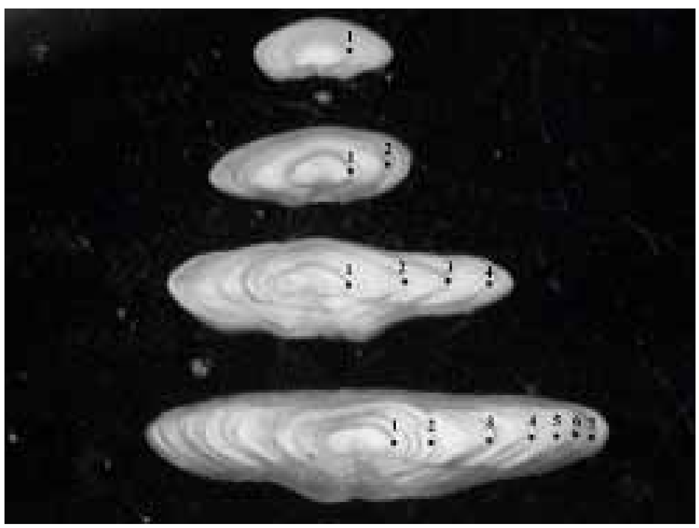


Fig. 12. Comparison of otolith sections from fish of different sizes. The otolith on the top is from a 6.5 cm female (captured in November 2000), next otolith is from a 12-cm male (captured in October/November 1999), a 23-cm female and a 39-cm female (also captured in November 2000). Note the edges on the otolith sections, and in the last otolith note that an annulus has started to form but because it was captured in November it is not counted.

The reader should attempt to manipulate the light and angle of the otolith being examined, as this often ensures greater clarity of the growth pattern. It should be kept in mind that at all lengths and all ages, variation in size and age exists. However, until about 25 cm, age does not tend to vary more than ± 1 year for 5-cm length groups. For example, all five otoliths in the following photo (Fig. 13) are taken from 25-cm yellowtail flounder. Otoliths A, B and E are from females, and C and D are from males. Otoliths A, C and E were captured in July, B in May, and the fourth in November. They range in age from 4-5 years old. They were all captured in 1999-2000. However, in Fig. 14, the fish were captured in 1984-1985, and the fish tend to be older at length than in more recent years.

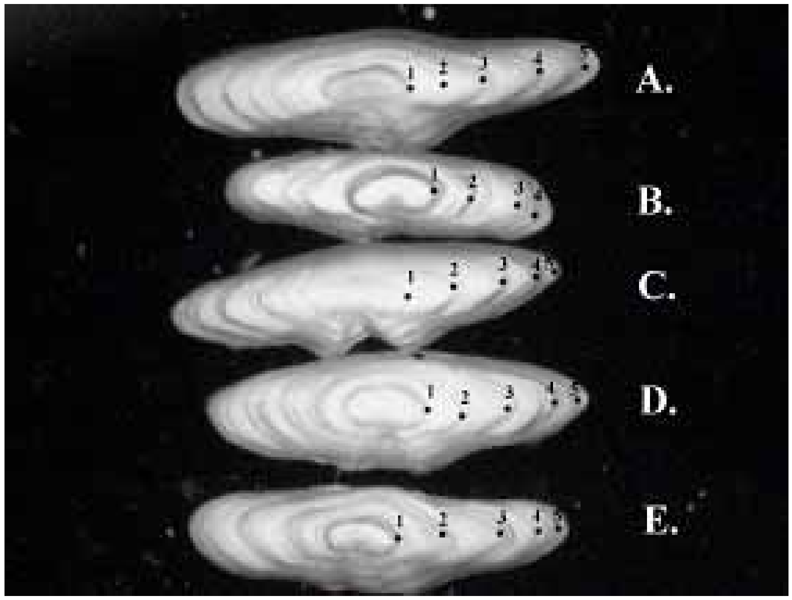


Fig. 13. Comparison of five yellowtail flounder otolith sections from 25-cm fish. They range in age from 4 to 5 years old. Otoliths C and D are from males, while the rest are females. All were captured in November 2000, except for otolith B, which was from a fish captured in October/November 1999. Because of the time of year capture, the otolith in Figure 13C would probably be aged as a 4-year old.

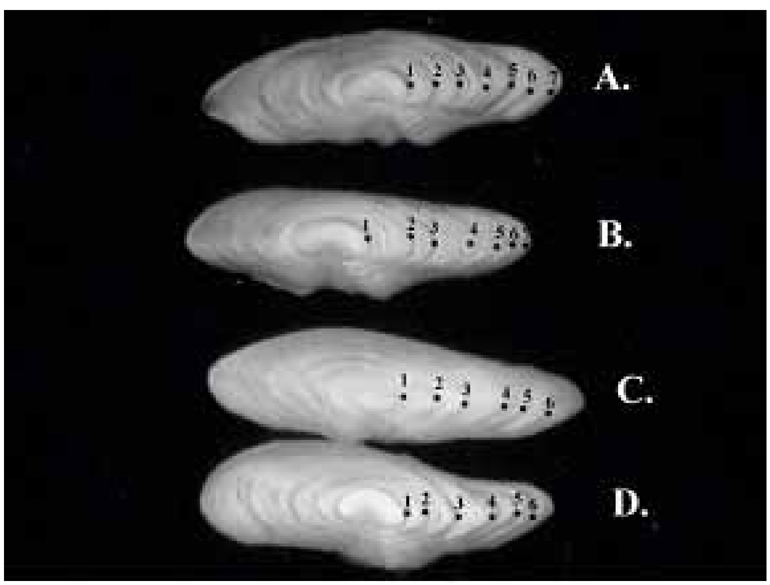


Fig. 14. Comparison of otolith sections from four yellowtail flounder from 25-cm fish. These fish were collected in 1984-1985 period and range in age from 6-7 years. Otoliths in A (male) and B (female) were collected in August 1984; otoliths C (female) and D (male) were collected from January 1985.

Whole Otoliths

Otoliths sampled from yellowtail flounder are stored dry in envelopes. Otoliths are not soaked in glycerin, they are simply removed from the envelope, placed in a black dish, covered with 95% ethyl alcohol (although water can be used) and examined under reflected light at magnifications of about 7–25 X.

Traditionally, the surface of whole otoliths were ground in order to enhance growth rings but it was found that there was no difference in the number of annuli read between ground and non-ground otoliths (Dwyer *et al*., MS 2001). Since we are now sectioning fish >25 cm, it is unnecessary to do this. However, if the reader feels it is necessary, there is no reason why the otolith should not be ground. If the otolith is to be used as a section later, though, it should not be ground as this removes some of the ageing material.

The distal surface of the left otolith is the side of the otolith most usually read. The proximal side contains the *sulcus acusticus*, a groove which runs down the length of the otolith and can obscure growth zones (Fig. 4). However, in some young fish, the proximal side of the left and right otolith is very clear and can be used for ageing. In very young fish (0–2 years), annuli are so clear, it does not matter which side of the otolith is used.

In general, yellowtail flounder whole otoliths are not clear (annuli are not easily visible). However, on occasion, some otoliths are found with very clear annuli, particularly in inshore yellowtail flounder (Fig. 15). In addition, otoliths from very young fish (0–4 years) tend to be fairly easy to read.

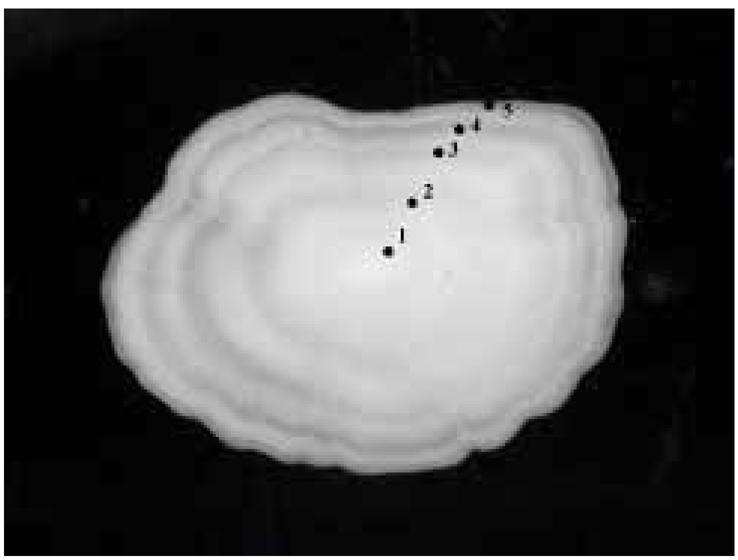


Fig. 15. Yellowtail flounder collected in April 1999, from Witless Bay, Newfoundland. It is a 5-year-old 21-cm female fish. Note that although the annulus is forming on the edge, it is still counted as a 5-year-old fish.

Annuli can be read along any growth axis, but should be followed around the otolith completely to make sure that it is an actual annulus, and not a check, and also to determine edge type (see Edge Type Section below). Often the rostrum (see Fig. 4) is the fastest growing area of the otolith and has a different amount of new growth showing than the rest of the otolith. When determining age, it helps to examine a number of different growth axes, but best to pick one for determining edge for consistency.

The term “crystallized” refers to otoliths composed of *vatarite*, a structural variant of aragonite (Forsberg, 2001). For yellowtail flounder, crystallized otoliths should not be read and cannot be thin-sectioned. It is unknown why this happens and may occur in one otolith or both. If they are partially crystallized, the otolith may be read, depending on the amount of crystallization, and an age assigned. Figure 16 shows two examples of crystallized otoliths, they are both shiny.


A.


B.

Fig. 16. A crystallized otolith from a 48-cm female yellowtail flounder (A) captured in August 1998. Figure 16B shows a crystallized right otolith from a 46-cm female yellowtail flounder, captured in October/November 1999. It is possible that the otolith in Fig. 16A could be read, but the otolith photographed in Fig. 16B would not be read.

As with thin sections, whole otoliths may be difficult to read and recognizing that a pattern of growth exists is often as important as reading individual rings. Sometimes a process of "lumping" is used to include units of closely spaced translucent zones that are separated by wider opaque zones as single annuli. Figure 17 shows a drawing of this situation. The photograph of the otolith in Fig. 18 demonstrates this.

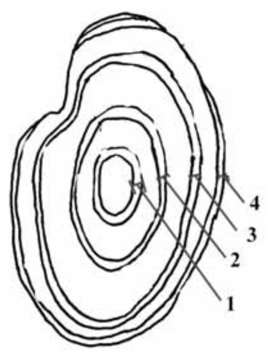


Fig. 17. Diagrammatic representation of a lumped pattern. Modified from Forsberg, 2001.

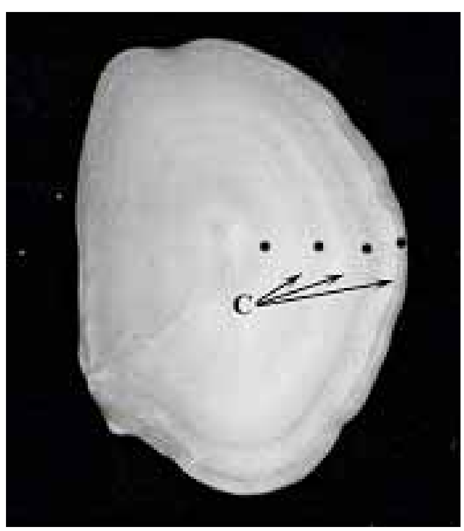


Fig. 18. This otolith may be 4 years old if one "lumps" the checks and annuli as seen in the photo, or if one splits and counts all apparent "annuli", one gets a count of 7 years. This otolith is from a 26-cm female yellowtail flounder and was captured in November 1987. The image is enhanced digitally with an unsharpen mask (Adobe Photoshop 5.0 LE).

When whole otoliths and sections are directly compared, it can be noted that whole otoliths have fewer visible checks than the section, but the annuli on the edge of the otolith are harder to see, especially in older fish (>6–8 years) (see Fig. 19–23). Figures 19–23 show a comparison between some whole and sectioned otoliths.

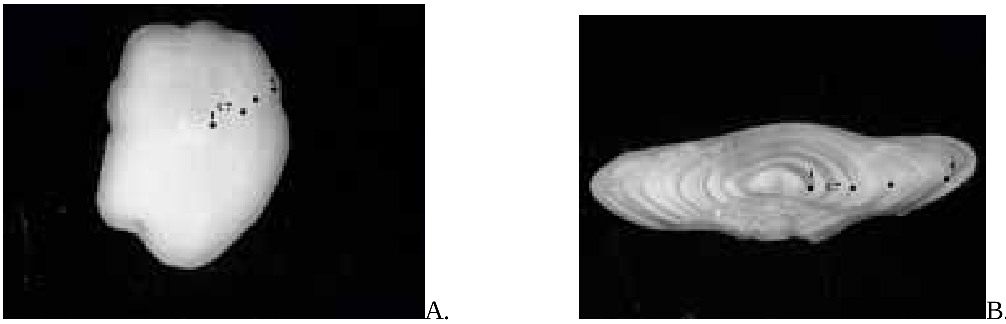


Fig. 19. Comparison of whole otolith (A) compared to sectioned otolith (B). This otolith was taken from a 19-cm female, which was aged as 4 years old. However it could also be considered 5 years old if the prominent check (marked 'C') is counted as an annulus. It was captured in June 1998.

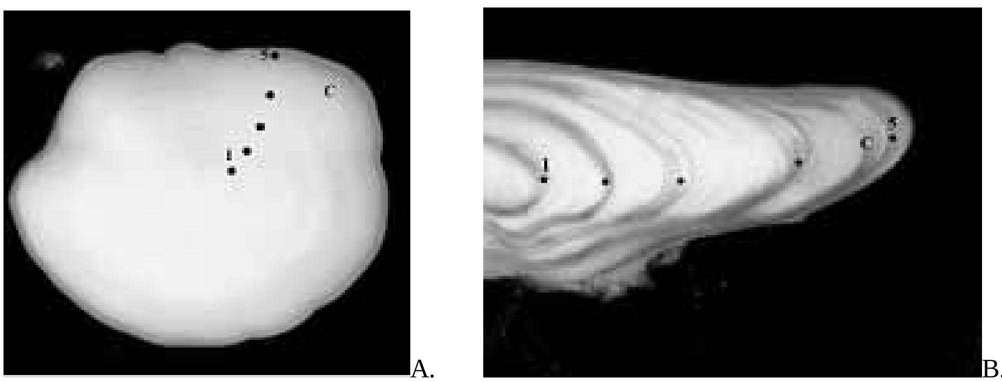


Fig. 20. Comparison of whole otolith (A) compared to sectioned otolith (B). This otolith was taken from a 23-cm female, which was aged as 5 years old. Notice that the patterns of growth are the same on the whole and sectioned otolith. It is unusual that the first three zones of growth are slow, and the next two faster. It was captured in June 1998.

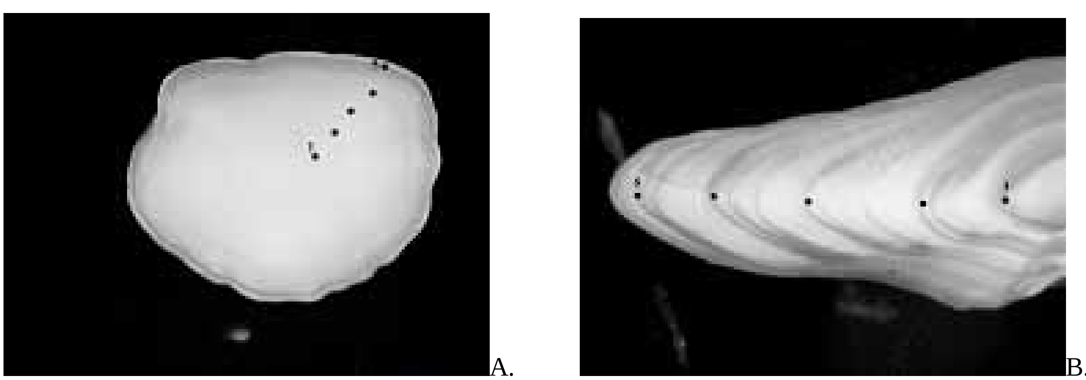
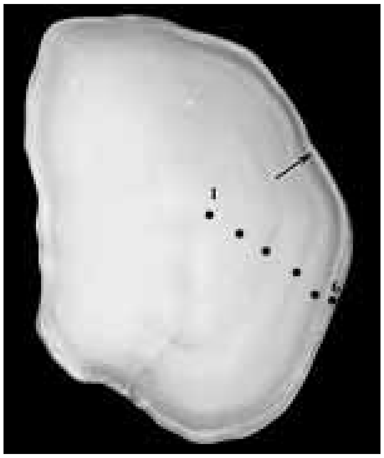


Fig. 21. Comparison of whole otolith (A) compared to sectioned otolith (B). This otolith was taken from a 25-cm female, which was again aged as 5 years old. It was captured in June1998.


A.

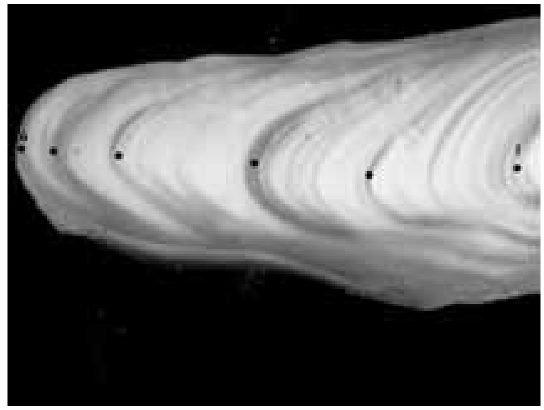
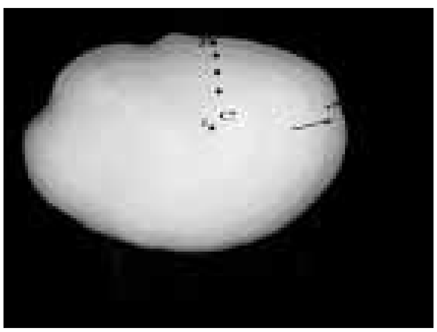
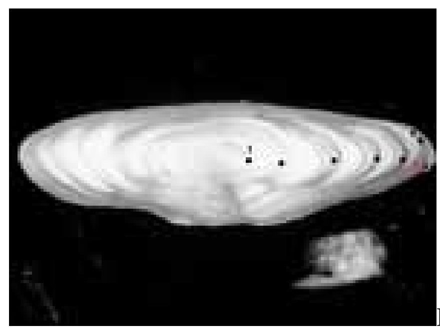

B.

Fig. 22. Comparison of whole otolith (A) compared to sectioned otolith (B). This otolith was taken from a 28-cm male yellowtail flounder, which was aged as 6 years old. The arrow points to an area on the otolith where the new annulus has not formed yet. If the otolith had been cut further up, it would have been difficult to see this new growth. Thus, it is often important to examine both the whole otolith and the sectioned otolith. It was captured in June 1998.


A.


B.

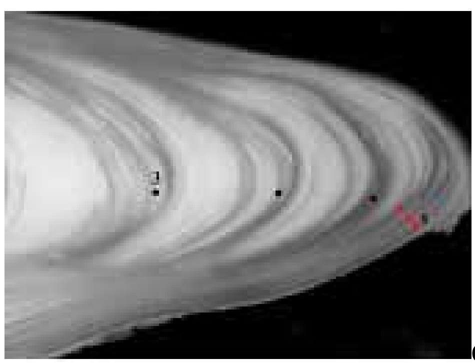

C.

Fig. 23. Comparison of whole otolith (A) compared to sectioned otolith (B and C). This otolith was taken from a 30-cm female yellowtail flounder, which was aged as 5 years old from the whole otolith. It can be seen from the area by the arrow, that there may be more annuli present. However, it is only when it is sectioned, three more annuli can be seen growing laterally on the otolith. It should be noted as well, that the second annulus is sometimes difficult to see on a whole otolith and may be missed. It was captured in August 1999.

Thin-Sectioning Preparation

Thin sectioning at NAFC follows the method outlined in Penttila and Dery, 1988, p. 9.

Supplies:

Isomet low-speed saw (Buehler 11–1280)
Diamond-tipped blades (Norton Co. through K & D Pratt, Nova Scotia)
F0565977 Norton Diamond Blade
3 x 0.006 x ½ Type 1A1
Blueprint ME 120929
M4D 10/29M1-N50M9-1/8

Dressing stones (K & D Pratt – distributor)
Paraffin Wax
Marking tags (Avery marking tags 1 ½" x 15/16")
Carbon, decolorizing
Calcium oxide
Double sided tape
Hot plate

Step 1: Embedding Mixture

Melt wax on hot plate, and add to mixture, approximately 4 parts melted wax, 3 parts calcium oxide and 1 part carbon. This mixture should not be too runny but should not be too thick either. The mixture is cleaner if the carbon is not used, but the otolith is harder to see. It is up to the processor what to use.

Step 2: Marking the tags

Pencil a crosshairs on the tag, ensuring that the centre lines up with the blades when placed in the chuck. This will be used to ensure the nucleus of the otolith is sectioned. Place a piece of double-sided tape on the tag before a drop of the embedding mixture is dropped onto the crosshairs of the tag. The otolith is then carefully oriented into the mixture by placing the sulcus along the shorter line, with the nucleus lined up with the centre (Fig. 24).

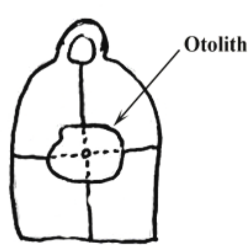


Fig. 24. Diagram of the dorso-ventral placement of the otolith on the tag for cutting.

The cut made is a dorso-ventral section. This step is very important. If the otolith is not oriented correctly, then the cut will not go through the nucleus, affecting the accuracy of the age reading.

The otolith is then covered with a layer of the embedding mixture. The entire otolith should be covered adequately, but do not use too much wax, as this takes more time for the saw to cut.

Step 3: Using the Saw

The blades are fairly delicate and should be treated as such. They should not be left on the saw overnight and cleaned daily. When not in use, the blades should be laid flat. The tray should contain cool distilled water (don’t use tap water, the tray is made of aluminum and corrodes easily). Add a few drops of detergent.

The size of spacer between the blades is up to the operator of the saw and the objectives of the sectioning process. There are a number of spacers that can be used. An old blade that has been fitted can be used as a spacer, thin plastic and Mylar can also be used. For regular ageing of yellowtail flounder otoliths, about 0.3–0.5 mm is appropriate. The thinnest sections tend to get broken and lost easily. Calipers should be used regularly to measure the section and ensure it is the suitable thickness.

Step 4: Place the tag into the chuck holder.

The crosshairs should be lined up with the blades to make sure the saw is cutting correctly. Do NOT lay the chuck and saw arm on the blades if the blades are not moving (as this may warp the blades). Then, holding the chuck above the blades, begin the rotation of blades in mid-cycle. Then slowly and gently lower the chuck and otolith onto the blades. The rotation should be turned up to maximum.

The saw can be set so that an automatic shut-off switch stops the cutting when it has cut through the section. The tag should be removed and the section taken out.

Step 5: Materials for Holding the Section

The otoliths are stored in folded paper. Small pieces of dark construction paper can be placed at the bottom of this fold so that the otolith is easier to see. This can then be placed, along with the two pieces remaining on the tag, into an envelope. This can be a problem, as the section is loose, and therefore hard to handle.

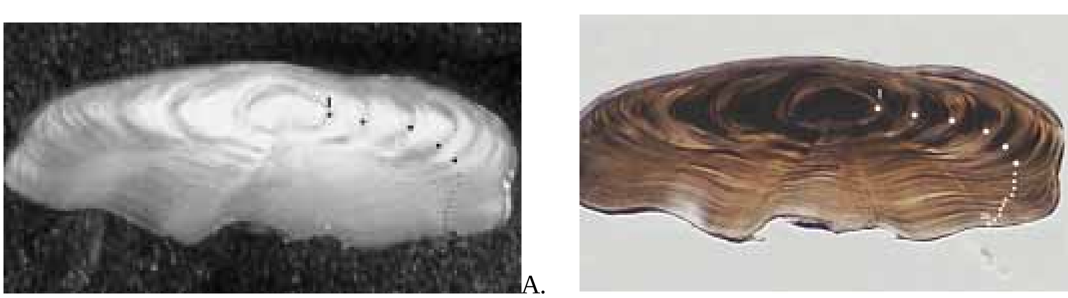
Step 6: The blades

The blades should be cleaned daily by washing gently with hot water and detergent and then rinsing. After a prolonged period of time, the blades will need to be dressed. There are special dressing stones for the Norton blades, which should be fitted tightly into a special chuck. Blades should be run through one at a time (not double) and about 5–7 times for each. Allow the blade to run through about ¼ cm (not past the diamond tipped edge of the blade). Lay the dressing stone in the chuck down slowly, as the blade is rotating (usually at 2–4 rpm).

Reading Thin-Sectioned Otoliths

Otoliths are sectioned using a low-speed saw and taking a section approximately 0.4 mm thick (see Thin-Sectioning Preparation Section). Where possible, the left otolith is used but if not present, the right otolith can be sectioned, taking care to section directly through the nucleus. If possible, it is beneficial to the reader to section the left otolith and keep the right otolith for comparison even in older fish. This section is stored in a fold of paper and in an envelope. Sections can be examined under either transmitted light or reflected light, but in most cases reflected light is adequate, ethyl alcohol is used to cover the section, and observed under 25–40 X magnification with a dissecting scope but may be examined for checks and other unusual anomalies under lower power (7.5–20X).

Sectioned otoliths reveal an opaque inner region followed by alternating translucent and opaque zones, which under reflected light, appear dark and light, respectively. However, it is important to remember that the opposite is true if examined under transmitted light (see Fig. 25) as mentioned.



A. B.

Fig. 25. Yellowtail flounder otolith section examined under reflected light (A) and transmitted light (B). This section was taken from a 46-cm female, and was captured in November 1998.

Under reflected light, age is estimated as the number of completed translucent zones, although a full year of growth is one opaque plus one translucent zone, as in whole otoliths.

Annuli *versus* Checks

Similarly as with whole otoliths, annuli and subsequent opaque zones are large between 5–7 years, after which there is a considerable narrowing of annuli. Each annulus is distinguished by being continuous (can be in all growth axes of the section), and usually dark in comparison with checks. Knowledge of typical growth patterns helps to distinguish annuli from checks. Checks are defined as discontinuities in the opaque zone. They are assumed to be stress-induced. Checks may be discontinuous, irregularly spaced and/or very narrow. They may be vague or diffuse and in general, not consistent with the growth pattern. Checks are clearly seen under high magnification, and reducing the magnification tends to increase the ability to differentiate between annual and sub-annual zones (checks). For example, in Fig. 26A at a magnification of about 30X, the potential check (‘C’) appears strong and continuous (although when traced to the sulcus it looks as if it joins with the annulus). However when examined under lower power (7.5X) in the next figure, it appears this is a check because annuli, as stated, do not form so closely together in the younger years. This should not be used to determine annuli from checks in older fish however, because it is difficult to distinguish all annuli present under low power magnification. Notice also how difficult it is to detect the annulus at the edge of the otolith under low power.

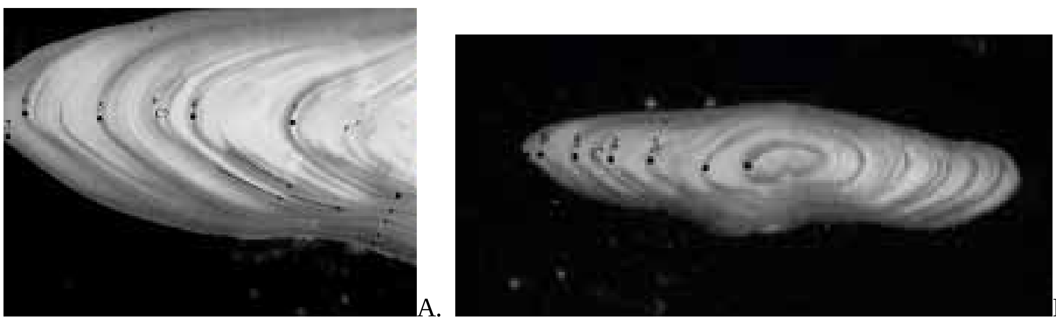

A. B.

Fig. 26. Examples of checks seen at high and low magnification of the same otolith from a 32-cm female yellowtail flounder. It was captured in November 1999.

In addition to environmental stress, checks may also be caused by maturation, spawning or metamorphosis. Figure 27 shows an otolith from a fish which might display a “spawning check” (SC).

Fig.27. An example of a check (C) and possible spawning check (SC) which cannot be traced into the sulcus area (discontinuous). From a 33-cm female yellowtail flounder, captured in July 1999. The check between years 4 and 5 might possibly be a spawning check.

In Fig. 28, it is difficult to tell whether the fifth translucent zone is an annulus or a check. However if the fifth zone is traced into sulcus region, there is a vague continuation into the sulcus. Therefore this "check" would be counted as an annulus and the fish would be aged as 8 years old.

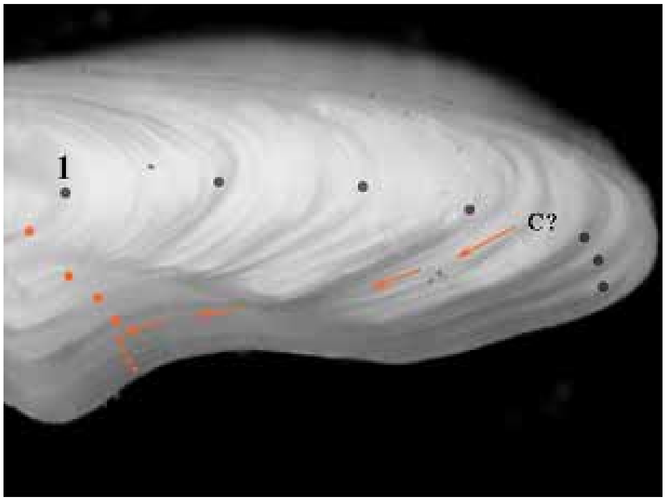


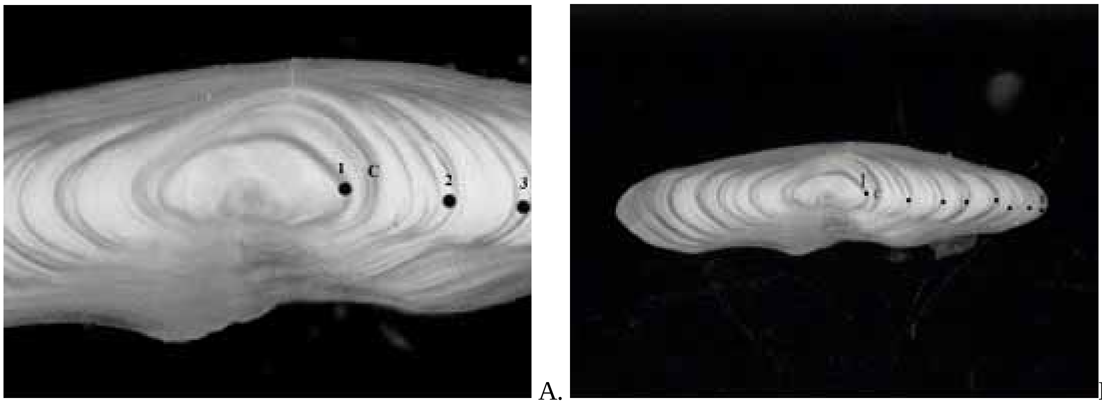
Fig. 28. Example of a potential check in a sectioned otolith from a 44-cm male yellowtail flounder. It was captured in October 1981.

First annulus

The first annulus is typically small (on average 0.71 mm in diameter) but distinct, which helps to determine which translucent zone is the one to start counting from, although it is important to note that there may be variation in size due to a prolonged spawning period.

In some cases, thin sections reveal a strong check outside the first annulus, which may actually be the second annulus (Fig. 29). This should be considered a check because the two rings (year one and 'C') nearly merge in the proximal axis. However, this check is extremely prominent and it is possible that calling it a check might be a misinterpretation, although it is fairly certain through length-frequency analysis that this ring is too close to the first annulus to be considered the second annulus (and is therefore a check). Therefore, it is presently considered to be a check. If the second apparent zone is strong and can be followed into the sulcus, it is counted as an annulus, but some judgement should be used. Typically, the situation is such that the first five annual zones are wide (see Fig. 21B). However, in the "small second annulus" situation, the second annulus looks very close to the first annulus (Fig. 29).

Sometimes the first annulus is unusually small or the distance between annuli 1 and 2 are smaller than between 2 and 3. This might be because the fish was spawned at the later end of the spawning period (later in summer) and had a short first growing season, as is seen in Pacific halibut *Hippoglossus stenolepis* (Forsberg, 2001), for example. Some first annuli appear round in cross-section, but others may be oval as well. The third to fifth annuli tend to be more pointed and "stretched-out" along the longest growth axis than the first or second annuli.



A. B.

Fig. 29. It is unlikely that the "second annulus" (C) is a true annulus and is more likely a strong check. This is a 27-cm female yellowtail flounder captured in January 1985.

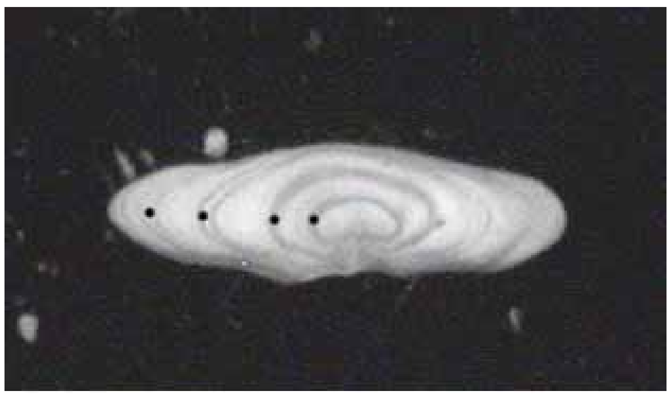


Fig. 30. A four-year-old 18-cm male yellowtail flounder, captured in November 2000. The second ring would probably be interpreted as an annulus, because the spacing is further apart than the spacing between the two first rings in Figure 30. This could be an example of a late spawner.

Old Growth

In very old fish, otoliths cease growing in length, but continue in depth, so that annuli should be examined more closely in the area between the fastest growth and in the sulcal region (Axis III from Fig. 7). Figures 31 and 32 are examples of the lateral growth than continues in otoliths from older yellowtail flounder (>7-10 years).

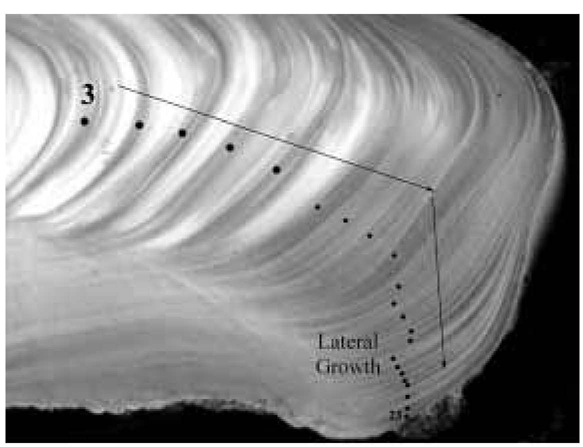


Fig. 31. An example of the lateral growth that exists in otolith sections from older yellowtail flounder. This fish was recorded as a 33-cm female yellowtail flounder that was captured in July 1999. It is unexpected that a fish of this size would be 23 years old, there might have been a mistake in recording the length on the envelope label.

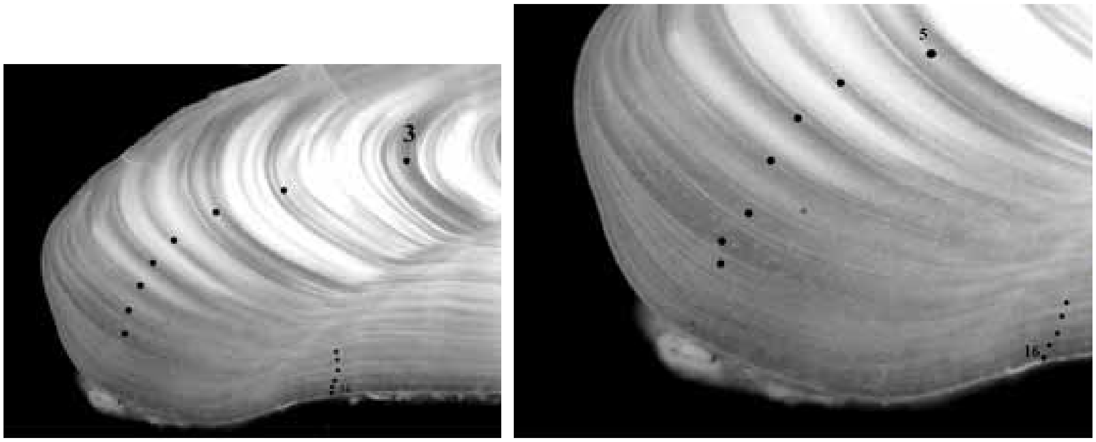


Fig. 32. Another example of lateral growth in a sectioned otolith from a 40-cm male yellowtail flounder, captured in October/November 1999.

In addition, there is a shift in pattern in many older fish, and in some cases, it seems the annuli “bunch together” with a number of years joined and then resume a typical pattern later. Figure 33 shows an example of this, which tends to be fairly common in yellowtail flounder. Note also a potential spawning check in this figure.

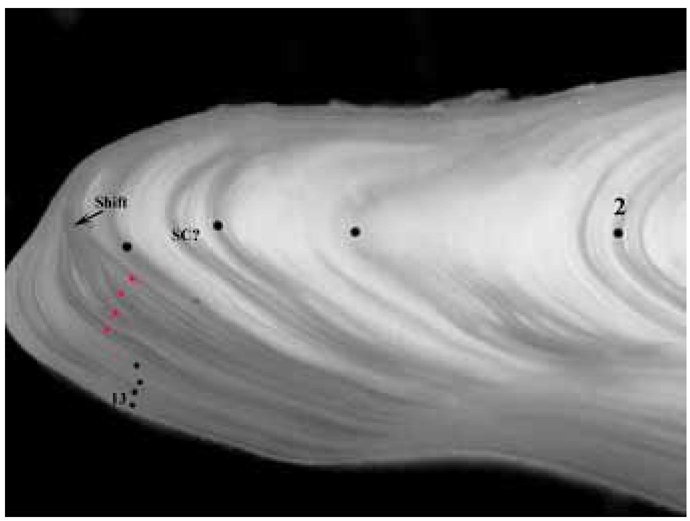
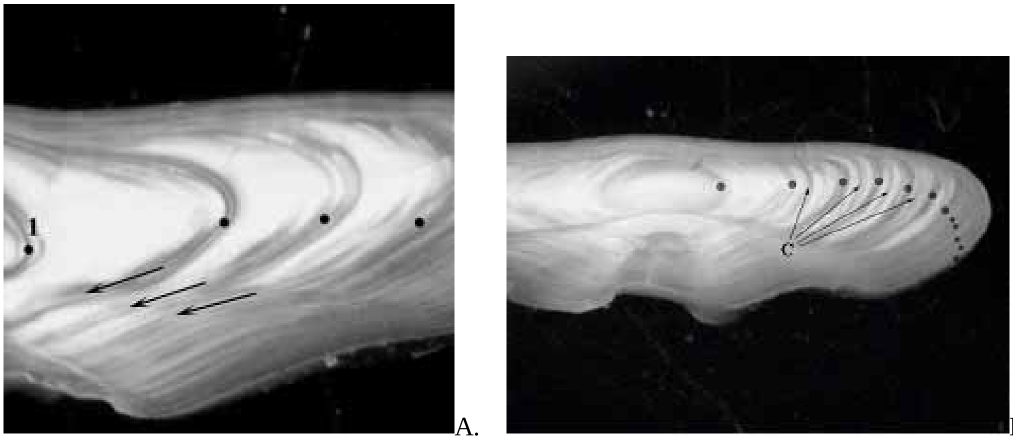


Fig. 33. Example of a “shift” pattern that shows several years bunched into one. This otolith is from a 50-cm male yellowtail flounder. It was captured in November 1988.

As with whole otoliths, at times, there is what appears to be a “doubling” or “splitting” of annuli. At high magnification these can be seen and even traced back to the sulcus (Fig. 34A and B). Efforts should be made to recognize these patterns and ensure that both rings are not counted. Figure 35 shows an image of a ‘split’ first annulus.



A. B.

Fig. 34. There appears to be a “doubling” or “splitting” of the annuli in some samples. This may be a problem in older fish when it is more difficult to separate the annuli. The otolith in panel A is from a 42-cm male, captured in October/November 1999 and the “doubling” can be seen in the sulcus area. In panel B, the otolith is from a 52-cm female yellowtail flounder captured in May 1983 and it appears as if there are a number of “split” annuli. Knowledge of growth patterns can help a reader determine whether a ring is a check or an annulus.

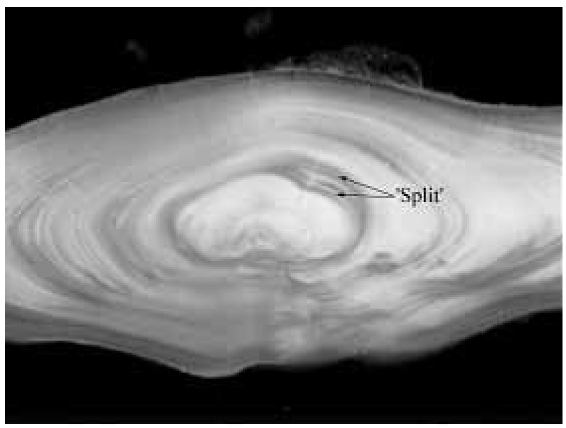


Fig. 35. An otolith section from a 27-cm female yellowtail flounder showing a ‘split’ first annulus. This fish was captured in June 1998.

Annuli may be composed of numerous translucent zones or checks forming close together (called “checky”) (see Fig. 36) causing true annuli to be difficult to distinguish, especially in older fish. In these cases, the term “lumping” can be applied, where many checks are “lumped” and counted as one annulus.

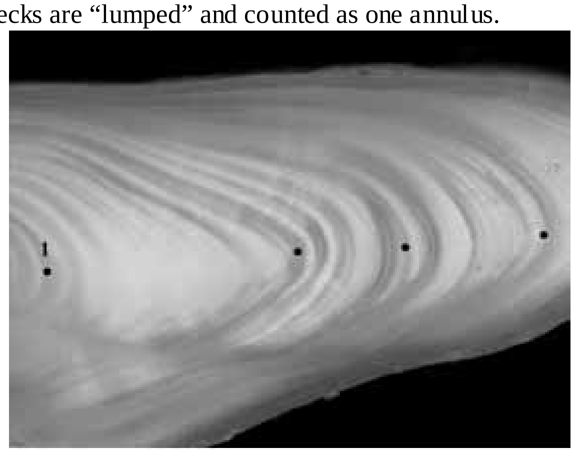


Fig. 36. An example of a “checky” otolith. This otolith is from a 22-cm female yellowtail flounder captured in November 2000. The checks seen here would be grouped or lumped together to be considered one annulus.

In some cases, an annulus is vague or not easily identified, but due to the overall annual pattern of growth zones, a reader should recognize that an annulus is probably present. This is the case in Fig. 37.

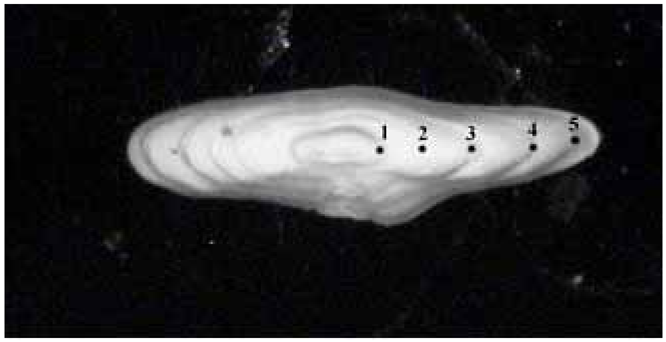


Fig. 37. This otolith was taken from a 25-cm female yellowtail flounder, captured in July 1999 and was aged as a 4-year-old. The second year is vague, but can be seen better on the ventral side of the otolith.

Knowledge of typical growth patterns is important. Sometimes, in addition to weak annuli being present, there are two annuli spaced closely together and because they appear prominent and continuous, they are counted as 2 years, but the growth pattern is unusual. This may be called "splitting", or a "split annulus". It is often hard to distinguish the annulus from the check. An example of this is found in Fig. 38.

Fig. 38. A 32-cm female yellowtail flounder captured in November 2000. Note that year 4 is vague but that if was not counted, the pattern of growth between 3 and 4 would be too wide. In addition, when the weak 4th year is followed into the sulcus, it looks more prominent. Between years 5 and 6, however, although spacing is irregular between these years, they would both be counted as annuli. This is an example of the rare situation in which the growth increment between two earlier years (5 and 6) are less than between older years (6 and 7).

There are some otoliths which have an unusual shape (Fig. 39). The sulcus area of these otoliths is more expanded and more well-defined than seen in the typical otolith and annuli can be counted from the sulcus area (Axis III).

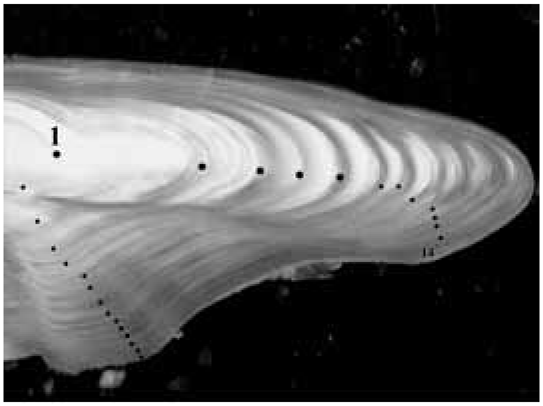


Fig. 39. An example of an otolith with an unusual shape. This otolith was taken from a 47-cm female yellowtail flounder, captured in October/November 1999.

Validated Images

The following images have been validated using bomb radiocarbon assays (Dwyer *et al.*, 2003) and can serve as a guide for age determination of sectioned otoliths (Fig. 40-42). The images below are enhanced digitally with an unsharpen mask (Adobe Photoshop 5.0 LE). These can be found on the Otolith Research Laboratory, Bedford Institute of Oceanography: http://www.mar.dfo-mpo.gc.ca/science/mfd/otolith/english/new_page_1.htm.

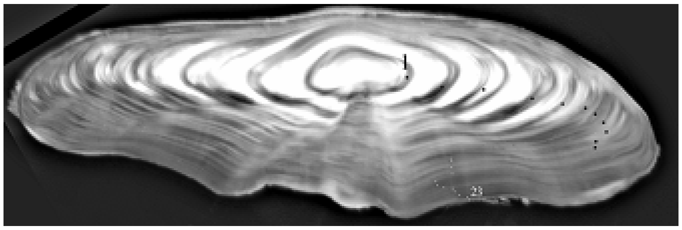


Fig. 40. A sectioned otolith from a 51 cm female yellowtail flounder that was aged at 23 years of age. The fish was captured in April 1980.

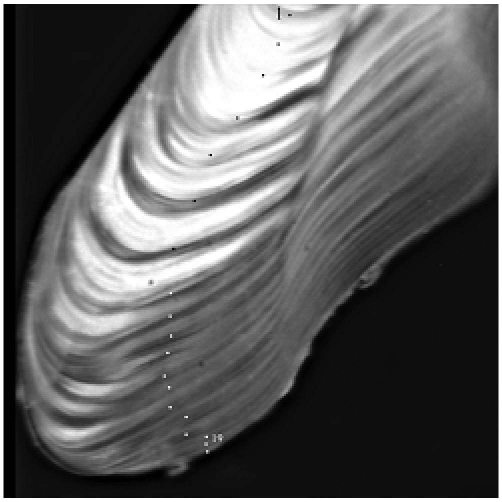


Fig. 41. A sectioned otolith from a 50 cm female yellowtail flounder that was aged at 19 years of age. The fish was captured in October 1972.

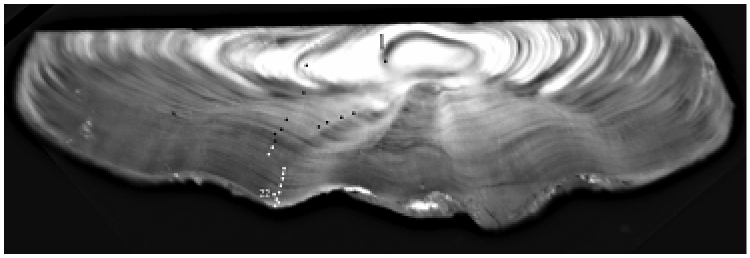


Fig. 42. A sectioned otolith from a 54 cm female yellowtail flounder that was aged at 22 years of age. The fish was captured in October 1972.

Edge Type

Edge type is important for determining the year in which the annulus has formed (age designation). Typically the annulus is finished forming in spring (March–April) in cold water flatfish, and opaque growth forms throughout the summer and fall of that year. Then the annulus begins again during the winter, although we suspect that very little somatic growth occurs over the winter. There can be a lot of variation in the type of growth at the edge of an otolith, and therefore the area where edge growth is interpreted should be consistent. Edge growth is usually classified as Narrow Opaque, Wide Opaque, Narrow Translucent or Wide Translucent and should be compared to the amount of material that has been laid down in the previous year. Typical edge growth is described in Fig. 43.

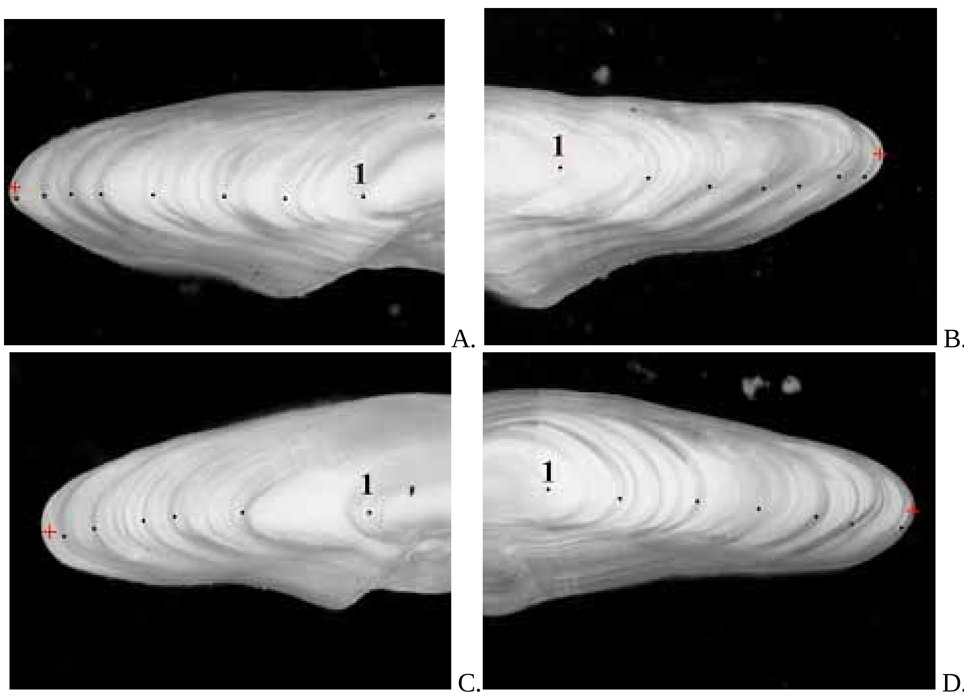


Fig. 43. Otolith sections showing edge growth for the four seasons. Although there is variation in each season, the otolith edge growth tends to be as in the figure. Red crosses indicate the edge type of each otolith. Panel A shows and otolith from a 25-cm female fish that was captured in February 1985 and is continuing the formation of winter growth. It is classified as having a wide translucent edge. Panel B shows an otolith from a 25-cm male yellowtail flounder, captured in April 1985. The annulus has just finished forming and a small amount of opaque edge has been deposited. Its edge is classified as narrow opaque. Panel C shows an otolith from a 26-cm male and this fish was forming its summer zone at its time of capture in August 1985. Its edge is classified as wide opaque. Panel D shows an otolith from a 28-cm male captured in October 1985 and this fish is in the process of starting to form winter growth. It is classified as narrow translucent.

Inshore Yellowtail Flounder Otoliths

In general, inshore otoliths tend to be clearer and have wider annuli than offshore yellowtail flounder otoliths. Inshore otoliths seem to have a more scalloped edge (Fig. 44).

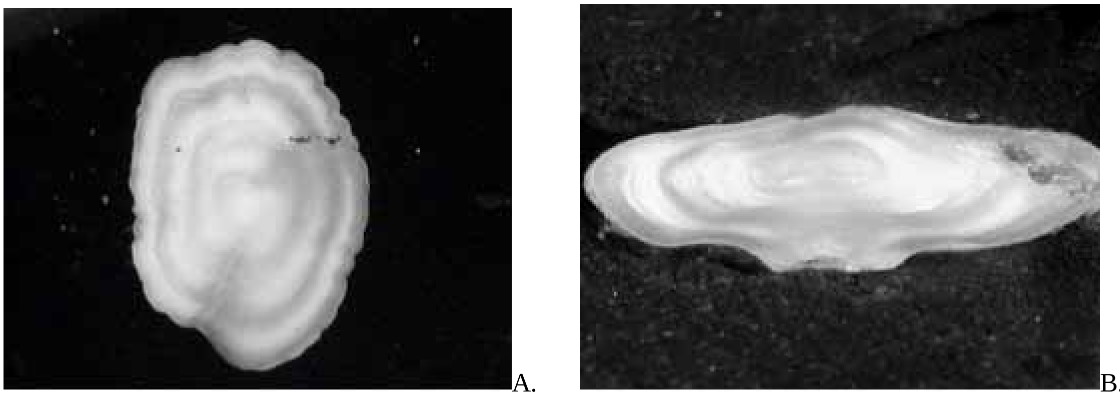


Fig. 44. An example of an inshore yellowtail flounder otoliths from a 14-cm male fish (A). Note the wide annuli and scalloped edge. The wide annuli can be seen in the section as well (not from the same otolith). Panel B is from a 12.5-cm female fish. Both fish were captured in April 1999 in Witless Bay, Newfoundland.

In addition, inshore yellowtail flounder seem to have slower growth than the offshore fish, but only a small number of otoliths have been examined (Fig. 45).

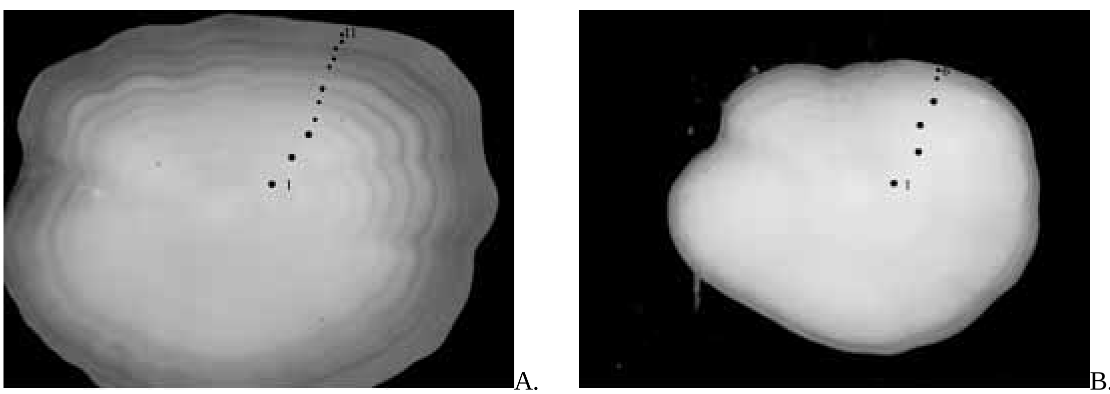


Fig. 45. Both otoliths are from 30-cm yellowtail flounder females and each photograph was taken under 7.5X magnification. The otolith in Panel A was collected from inshore yellowtail flounder in April 1999 and the otolith in Panel B was collected from an offshore (Grand Bank) yellowtail flounder captured in October/November 1998. The whole otolith age from the inshore fish was 11 years old, while the otolith from the offshore fish was aged at 6 years old.

Troubleshooting the Thin-sectioning method

There are some problems using the thin-sectioning method. Some areas, when sectioned, appear “cloudy” and annuli within that area are easily misinterpreted (Fig. 46). This happens most frequently in the sulcus area.

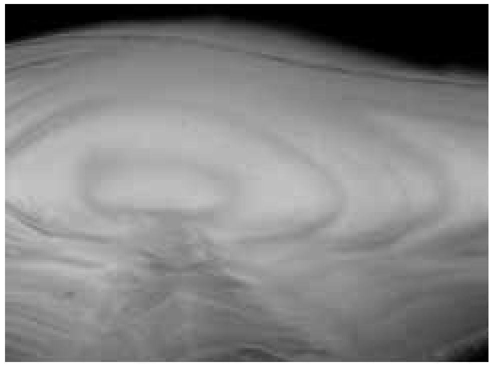


Fig. 46. An example of a “cloudy” area on a section. Sometimes this is in an area which obscures the reading of the annuli. This otolith was taken from a 49-cm female yellowtail flounder and captured in August 1988.

If the otolith is not lined up directly through the nucleus, the cut will miss the first annulus and it will either not be counted or it will be estimated. The series of cuts in Fig. 46 shows how an otolith section looks when it is not cut through the nucleus, and when it is cut directly through the nucleus.

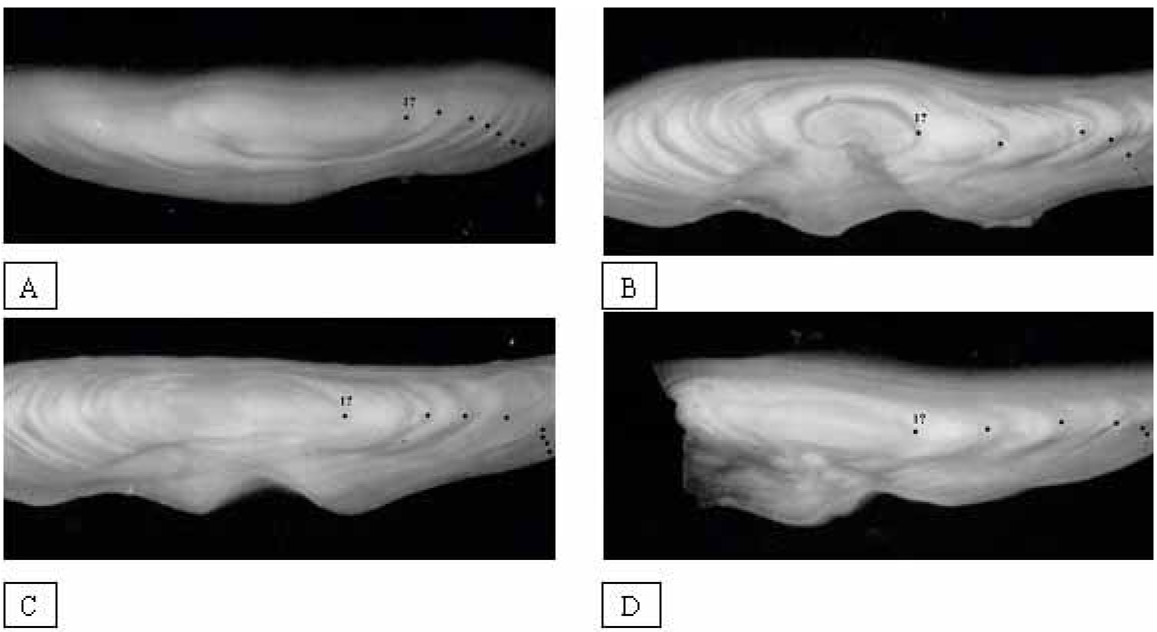


Fig. 47. A series of sections taken through the otolith. Note that only one of the series was cut through the nucleus (Panel C). Because of this, it is possible to miss the first annulus, and as a result, will have an inaccurate count. This otolith was taken from a 44-cm female yellowtail flounder and was captured August 1999. This should not pose a problem in most cases because it is a matter of knowing the pattern when the otolith is cut correctly and the resulting annual zonal relationships. Thus, for example, in Panel D, a reader should be able to tell that one annulus cannot be seen and adjust accordingly.

Conclusions

Ageing yellowtail flounder from the Grand Bank is accomplished by ageing whole otoliths for fish up to 25 cm and for fish >25 cm, thin-sectioning the otoliths. Like other fish species, young fish tend to have large annular growth zones, with wide opaque zones, although they usually have more checks. However, as fish get older (>7 years), the annular zones narrow and there is little, if any opaque zones between annuli. Often it is difficult to read the otoliths of the very oldest fish, even using thin sections, age may be underestimated; however it is the most accurate method at present.

Knowledge of typical growth patterns of yellowtail flounder is important for ageing this species and aid the reader in assigning an age class for each fish.

Acknowledgements

Special thanks to Shayne MacLellan of the Pacific Biological Station, Don Power and Dave Reddin, Northwest Atlantic Fisheries Centre, for reviewing this manual. Also thanks to Peter Upward for preparation of the samples.

Glossary

(modified from Penttila and Dery, 1988 and Walsh and Burnett, MS 2001)

Accuracy – the closeness of a measured or computed value to its true value.

Annulus – any zone which forms once a year, usually the "winter" growth zone which marks the end of a year of growth.

Annual growth zone – a growth zone that consists of one opaque zone (summer) and one translucent zone (winter).

Check – a discontinuity in a zone, or in a pattern of opaque and translucent zones.

Crystallized otolith – an otolith displaying inadequate calcification. Age determinations are generally not possible due to missing annuli.

Double annulus – discontinuity in an annulus, analogous to a "check". This causes the annulus to appear as two or more closely spaced "winter" zones.

Edge – outer periphery of the age structure.

Edge type – summer/winter or opaque/translucent deposition occurring on the outer edge of the age structure representing the most recent growth.

Nucleus – central portion of an otolith.

Opaque zone – a zone that inhibits the passage of light. With transmitted light, opaque zones appear dark; with reflected light, they appear white. "Summer" zones are composed of opaque material.

Precision – the closeness of repeated measures of the same quantity.

Reflected light – light from above used to illuminate objects below it.

Sagittae – Largest of three pairs of otoliths located in the sacculus of the inner ear of the fish; referred to as "otoliths" normally.

Settling check – characteristic check ring on some marine groundfish otoliths. It occurs just outside the nucleus and is believed to form during metamorphosis.

Spawning check – characteristic check ring formed when a fish, usually female, first becomes mature and spawns.

Split annulus – discontinuity in an annular zone, analogous to a “check”. This causes the annulus *at the fastest growth zone* to appear as two closely spaced “winter” zones or a split.

Sulcus acusticus **–** longitudinal groove extending down the proximal surface of an otolith (called the “sulcus”).

Transition zone – a region of change in otolith growth pattern between two similar or two dissimilar regions

Translucent zone – a zone that allows the passage of greater quantities of light than an opaque zone. Under reflected light and a dark background, the translucent zone appears dark and the opaque zone appears white.

Transmitted light – light from below an object used to shine up through the object for illumination purposes.

Validation - the process of estimating the accuracy of an age estimation method. Validation of an age estimation procedure indicates that the method is sound and based on fact.

Zone – region of similar structure or optical density. Synonymous with ring, band or mark.

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