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Yellowtail Flounder Ageing Manual

Karen Dwyer

2005

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Foreword

In accordance with its mandate to disseminate information on fisheries research to the scientific community, the Scientific Council of NAFO publishes the *Journal of Northwest Atlantic Fishery Science*, which contains peer-reviewed primary papers and notes on original research, and *NAFO Scientific Council Studies*, which contains review papers of topical interest and importance.

This issue of NAFO Scientific Council Studies contains a contribution nominated for publication by the Standing Committee on Publications (STACPUB) of the Scientific Council from research documents presented to its meetings. The 37 previous issues of the *NAFO Scientific Council Studies* are listed on the back cover of this volume.

May, 2005

Tissa Amaratunga, Editor
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Yellowtail Flounder (*Limanda ferruginea*) Ageing Manual

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Abstract

This is a technical manual describing the methods and interpretation used for determining age in yellowtail flounder (*Limanda ferruginea*). The paper gives a general overview of age determination, and discusses how age is presently estimated for yellowtail flounder at the Northwest Atlantic Fisheries Centre, St. John's, Newfoundland. The structure of the whole otolith is discussed, along with the limiting factors and constraints in the ageing of yellowtail flounder using this structure. Detailed information on the thin-sectioning method is given and how it is applied to the ageing of yellowtail flounder. The manual provides information on the types of validation studies that are used to ensure accuracy of ageing, and attempts to provide procedures for troubleshooting any difficult aspects of ageing. This manual contains a glossary and high quality photographs and diagrams to assist users when ageing yellowtail flounder.

Key words: ageing, otolith, thin-sectioning, yellowtail flounder

Introduction

Good quality age data from fish stocks is essential for fisheries biology, in order to estimate growth and mortality rates, maturity schedules and population age structure, and therefore is a vital part of the scientific process of stock assessment (Chilton and Beamish, 1982; Penttila and Dery, 1988). Ageing errors may create serious inaccuracies in stock assessments. Random error is usually not a problem, but systematic or bias errors are a concern because they lead to population dynamics miscalculations (Campana, 2001). For example errors can reduce differences in year-class strengths, leading to inappropriate yield projections from the stock assessments, and presumed high stock levels that could promote incorrect management (Chen, 1997). Both over- and under-ageing errors have an effect on fisheries management, but under-ageing errors are those that tend to produce advice that might lead to overfishing (Bradford, 1991; Lai and Gunderson, 1987).

There have been many methods employed to determine age of fish. Age of marine fish in temperate areas can be determined by counting periodic markings on a variety of "hardparts" of the fish. The term "hardpart" is general and refers to any part of the fish in which calcium or other substances are laid down periodically. For example, scales, vertebrae, spines and otoliths have periodic markings, which are presumed to be created annually in relation to growth and therefore have been used to determine age in fish.

The specific "hardpart" that is used to determine age in a fish depends on a number of factors which include water temperature, the latitude at which the fish is found, the rate of growth of the fish, the availability of fish to the sampler, the resources available to the sampler to collect and age samples, and the type of fish. The temperature (and usually latitude) at which a fish is found is related to its growth, and in general, fish that grow faster can be aged adequately with scales, whereas slower-growing fish living at colder temperatures and greater latitude should be aged with otoliths. With respect to the type of fish, elasmobranchs and some other species of fish that do not have otoliths and scales that grow throughout the life of the fish are aged using vertebrae or spines. In theory, all of the hardparts of a fish should return the same age, but that is not always the case. It is known that some hardparts are more reliable than others, especially in species where the fish reach old ages.

In general, age is estimated for fish from cold waters using otoliths. Otoliths are rock- or bone-like structures found in the balance organs of the fish located at the head of fish (also called "ear bones"). They are composed of calcium (Dannevig, 1956; Degens *et al.*, 1969). Once removed, otoliths can be prepared using several different methods, depending on a number of factors: they can be read whole, embedded in resin, polished, they can be broke in half and thin-sectioned, they can be burnt or stained or soaked in chemicals to enhance the rings.

The 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, which is the northernmost limit of the commercial concentration of this species, as well as southern stocks on the Scotian Shelf, Georges Bank and off Cape Cod.

A number of studies have looked at ageing of yellowtail flounder; for the southern New England stocks (Royce *et al.*, 1959), for Scotian Shelf stocks (Scott, 1947) and Grand Banks stocks (Pitt, 1974). For the stocks in the Northwest Atlantic, otoliths and scales have been commonly used to age this species. In the more southern stocks (e.g. Scotian Shelf, Georges Bank, off Cape Cod and Browns Bank), where stocks are faster growing, scales have traditionally been used to determine age of these fish for assessments. The fish from these stocks do not commonly reach as high a maximum age as other flounder stocks (about 7 years old (Penttila and Dery, 1988)). Northern stocks of yellowtail flounder, the Grand Banks stock, are aged using otoliths, as they are slower growing and until recently the age was determined using surface-read whole otoliths. However, tag returns from a 1990–93 Petersen disc tagging experiment (Morgan and Walsh, MS 1999) challenged Pitt's (1974) view of the age and growth dynamics of Grand Bank yellowtail flounder. Validation studies later indicated that thin-sections provided the most accurate ages for yellowtail flounder, even for the older fish (Dwyer *et al*., 2003).

This manual focuses on aspects of age determination for yellowtail flounder using both whole but especially thin-sectioned otoliths. Few manuals have been published on the ageing techniques of stocks from the Northwest Atlantic, although manuals exist for southern stocks of yellowtail flounder (Penttila and Dery, 1988) and for West Coast stocks of flounder (Forsberg, 2001; MacLellan, 1997). It is important that such manuals exist because age determination, by its very nature, is a specific science and methods and ages should be extremely consistent. Growth patterns are specific to a particular species in a precise area and therefore, ageing methods are modified to reflect this. In addition the manual serves as a model for those interested in setting up ageing labs or those looking to switch to a different method. Finally, this manual will serve as documented knowledge for new age readers and can be used in training.

Background: Materials and Methods

Otolith Characteristics

Whole otoliths, in general, are white, hard structures and in particular, flatfish otoliths tend to be somewhat flat, with an indistinct rostrum (a pointed end) and shallow *sulcus acusticus* (groove in the centre, running longitudinally) (Fig. 1). Yellowtail flounder otoliths are relatively small, ranging in length longitudinally from about 1–8 mm, depending on a number of factors, in particular the length of the fish. There are three pairs of otoliths located in the inner ear of the fish, the largest pair and the ones used for ageing purposes being the sagittal otoliths (Secor *et al.*, 1991). All flatfish have two different shaped otoliths; in family Pleuronectidae (the right eyed flounders, to which yellowtail flounder belongs) the left or dorsal otolith is 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). The opposite is true for left-eyed flounders. The unique symmetrical and asymmetrical sagittal otoliths in flatfish (Hunt, 1992), are due to the migration of the eye during metamorphosis and consequent growth of the fish in this position. A diagram of the left and right whole otoliths is seen in Fig. 2 and the left sectioned (Fig. 3A) and right sectioned (Fig. 3B) otoliths.

Otolith growth is related to the somatic growth (or growth of soft tissue) of the fish, that is, as the fish grows in body size so does the otolith. For the first number of years, as the fish grows rapidly, the otolith grows in wide opaque zones; however, after sexual maturity, when fish growth tends to slow, so does otolith growth, and in addition, tends to grow laterally (underneath) instead of ventrally and dorsally (out to the sides). Eventually, the opaque zones become more and more narrow until they are the same width as the translucent zones. Thus it appears in older fish like the annuli are compressed, and for that reason, may be difficult to interpret.

Otoliths 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 (see Glossary, page 15) 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). Growth in otoliths constitutes the deposition of hard tissue patterns where a year's growth consists of

Fig. 1. Photograph of left and right whole otoliths from a 40 cm female yellowtail flounder from the commercial fishery, viewed at a magnification of about 7–25×, and on the proximal surface. Otoliths range in size for yellowtail flounder from less than a millimetre to about 8 mm. The *sulcus* (groove) and rostrum (pointed end) can be seen in this image (see Fig. 2).

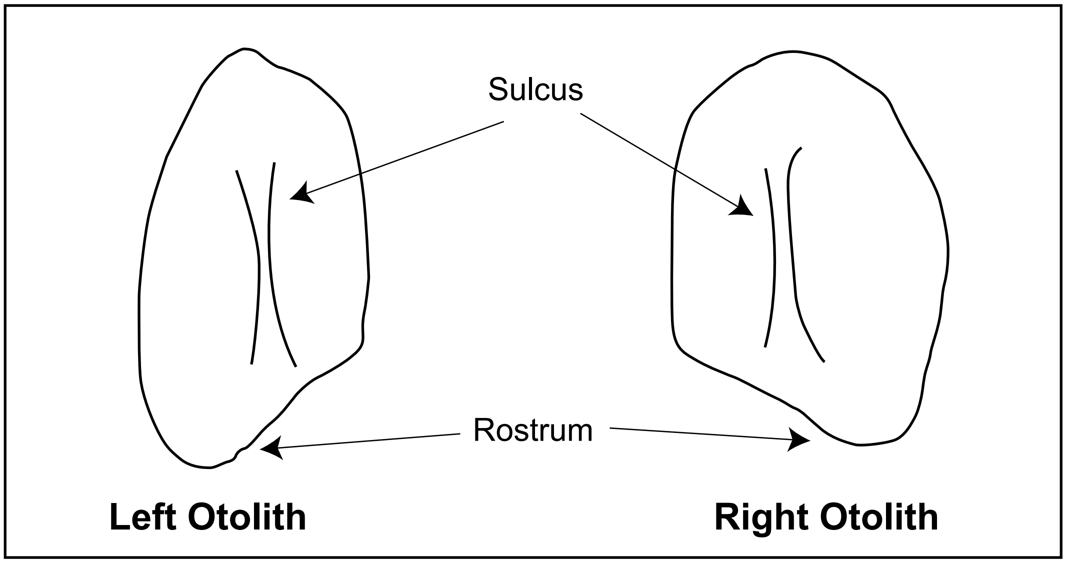


Fig. 2. Drawing of features of a whole otolith from yellowtail flounder, showing the proximal surface. The *sulcus* (groove) and rostrum (pointed end) of the otoliths are labelled.

one opaque zone and one translucent zone. This is referred to as an annual zone. Alternating opaque and translucent zones on the otolith are due to differences in protein deposition and the shape of aragonite crystals that are deposited in the otolith (Degens *et al.*, 1969; Penttila and Dery, 1988). The opaque zone is formed during the period of faster growth, usually in 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 illuminated. Opaque zones appear white under reflected light and the translucent zones appear dark (Fig. 4A) but the opposite is the case when the otolith is examined under transmitted light (Fig. 4B). Yellowtail flounder otoliths are usually examined under reflected light, so the terms "opaque" and "translucent" refer to white and dark zones, respectively. It is the "translucent" zone that is referred to as the annulus when seen under reflected light. Thin-sectioned otoliths can be examined under both reflected and transmitted light, but whole otoliths cannot be viewed with transmitted light as they are too thick.

Ageing at the Northwest Atlantic Fisheries Centre

At the Northwest Atlantic Fisheries Centre (NAFC), Department of Fisheries and Oceans Canada, Newfoundland and Labrador, yellowtail flounder up to 25 cm in total length are aged using surface-read whole otoliths and fish >25 cm are aged using thin-sectioned otoliths. Yellowtail flounder up to 25 years old have been aged using the thin-sectioning technique (the process whereby the whole otolith is transversely sectioned through the nucleus in order to get a cross-sectional view of the otolith).

There are, however, some problems associated with this method of ageing yellowtail flounder, including the interpretation of the first annulus, the "transition zone" (which is the area at which

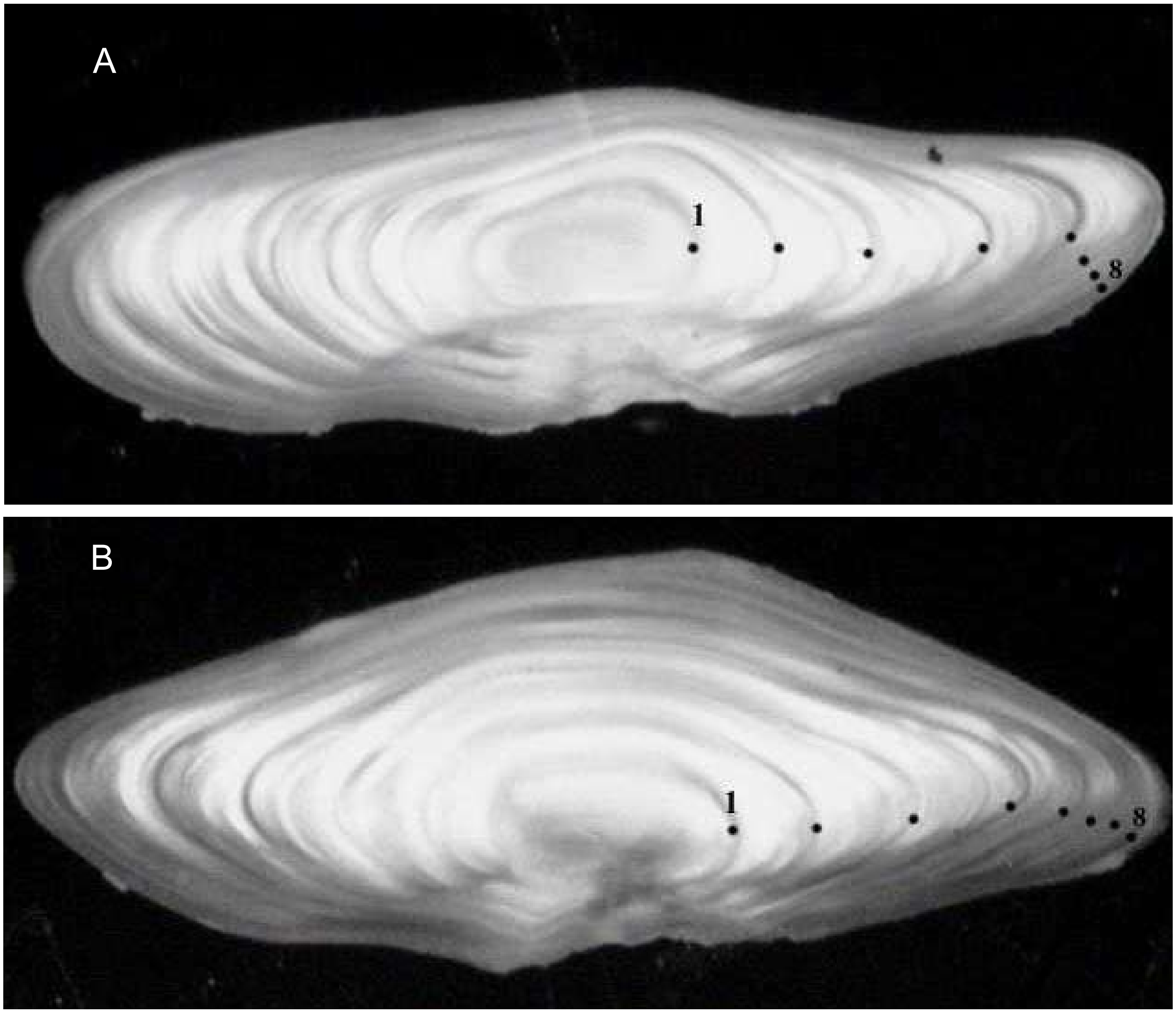


Fig. 3. Left (**A**) and right (**B**) sectioned otoliths from a 34 cm male yellowtail flounder captured in November 2000. Annuli are indicated by dots and numbers indicate the assigned age.

growth slows and switches from "juvenile" to "mature") and "edge growth" (which is the type of growth occurring on the edge of the otolith, and ultimately has to do with the age assigned to the fish). Annuli from the oldest fish are difficult to interpret.

In ageing methodology, accuracy refers to how close the estimated age is to the true age of the fish. Accuracy is determined by validating age methodology. At NAFC, both the whole (up to a certain age) and thin-sectioned otolith techniques have been validated using a number of techniques. The 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.*, 2003). Ageing thin-sections is the most accurate method for older yellowtail flounder, although even with this method it is difficult to distinguish between annuli at the very oldest ages. Like other fish, growth is faster in younger yellowtail flounder compared to older ones. As the fish

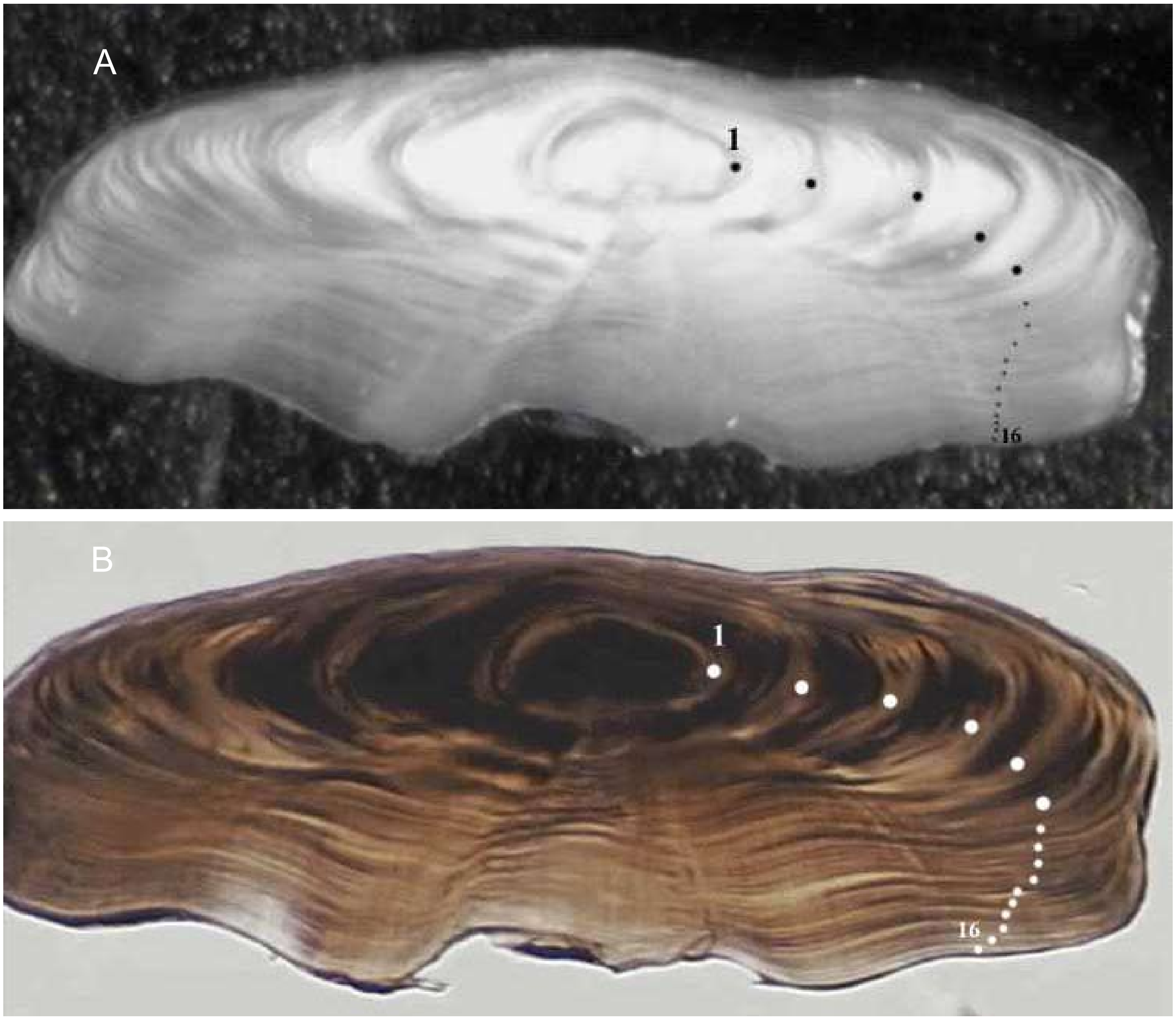


Fig. 4. Left sectioned otolith from a 46 cm female yellowtail flounder, captured in November 1998, under (**A**) reflected and (**B**) transmitted light. Annuli are indicated by dots and the numbers indicate the assigned age.

grows older, otolith growth is deposited laterally, and as a result, some annuli are not visible on the surface of the otolith. Therefore a thin cross-section is used to age the fish once it reaches a certain size (>25 cm). This technique is similar to one described in Penttila and Dery (1988), with some minor modifications (see Section 5 on Preparation for Thin-Sectioning Otoliths). Ageing with thin-sectioned otoliths has 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).

Collection of Otoliths

In the NAFC otolith collection program, sagittal otoliths are collected at sea twice a year, during the spring and autumn annual research vessel surveys. These otoliths are collected using a length- and sex-stratified random sample scheme (usually 1 cm/sex/set/strata); the detailed sampling protocol can be found in Doubleday (1981). The NAFC otolith collection program for yellowtail flounder also includes samples from commercial vessels which are collected in the same way as research vessel surveys, except by quarter, rather than season. Yellowtail flounder otoliths have been collected from research vessels since 1949 and commercial vessels since 1969. At sampling, both otoliths are usually removed from the fish head by locating the preoperculum and making a diagonal cut behind the eyes (Fig. 5). The otoliths lie one above the other.

The otoliths are collected and stored dry in envelopes for age determination back at the lab. Also recorded on the envelope are trip, set, date, fish length, sex, maturity and stomach contents. When otolith thin-sectioning is required, each section is placed in a small paper folder and returned to the envelope. This system of storage may change if a new mass-sectioning system is implemented, since in this system sections of many otoliths are made at once using a high speed saw, rather than a low speed saw doing one otolith at a time. When the mass-sectioning method is used, many otoliths are embedded in a resin block and all are sectioned at the same time. The slivers of resin containing the otoliths are then placed on large microscope slides and the thin-sectioned otoliths are coded and stored in this method. The mass-sectioning of otoliths promises to be more efficient with respect to collection and storage and a considerably faster method for preparing otoliths for age determination. This method has not been employed at NAFC at the time of writing, but it has been planned for the near future.

Technical Reading of Otoliths

A dissecting microscope is used to read both whole or sectioned otoliths. Otoliths are observed at magnifications from 7.5× to 50×, with reflected light from fibre optic light sources. The surface or whole otolith ageing is done using the lower end of magnification (7.5–20×), and the thin-sectioned

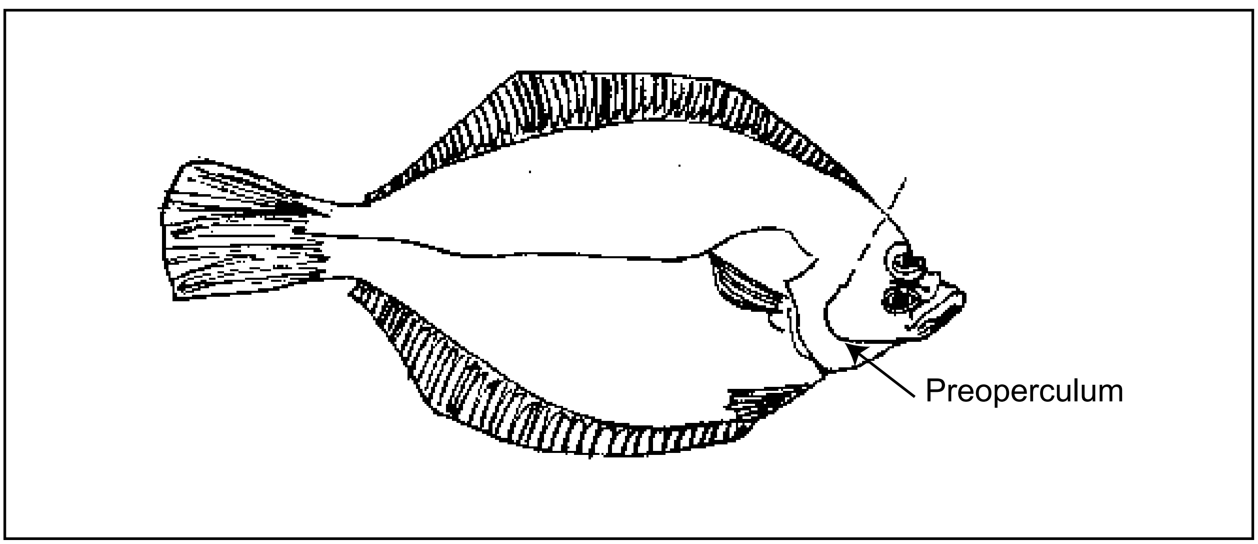


Fig. 5. Otolith removal from a flatfish; drawing of the yellowtail flounder shows a cut is made slightly behind the preoperculum diagonally across the top of its head, and otoliths collected from the *sacculus* inside the head (modified from van Helvoort (1986)).

otoliths are read using the higher end (25–50×). At the time of reading of the otoliths, the information provided to the reader from the envelope is time of capture and sex of the fish. Length of the fish is also given, although there are differing opinions on whether this may cause preconceptions in age determination. The reader records the age determined, along with edge type and reliability code associated with the reading. Reliability codes (Table 1) are not taken into account at the assessment level; however, they are used to check outliers in age-length keys.

Quality Control and Training

Quality control is an important issue that needs to be addressed by institutions that carry out fish ageing, particularly when ages are used for stock assessments. Quality control is meant to measure accuracy and precision among age readers. As mentioned, accuracy measures the 'true' age of the fish, while precision is the repeatability of age estimations, regardless of whether the age readings are accurate. At present a reference collection of otoliths for each Northwest Atlantic fish species is being considered at NAFC. This is a collection of otoliths considered to be representative of the fish in the stock across all seasons, length groups, sexes, areas, etc., for which a reliable age is known. If there are known-age otoliths in the collection, it may provide a valuable accuracy test for age readers. In addition, the reference collection can be used as a standard of precision age readings (Campana, 2001). This reference collection should be periodically re-read to measure within- and between-reader drift. The reference collection often serves as a valuable tool particularly if there is only one age reader. The collection can also be used for training. To ensure that changes in patterns over time are taken into account, recently collected otoliths should be added to the reference collection regularly.

The reference collection for yellowtail flounder at NAFC contains approximately 200 otoliths, including some known-age otoliths. In general the reference collection may be digital (as is some of the yellowtail flounder reference collection at NAFC), but if so, care must be taken to provide photos of several different angles at each magnification for each otolith, which is time-intensive.

As part of the quality control of age reading for flatfish at NAFC, readers are tested using age bias plots (to determine whether there is bias) and coefficient of variation (%) (*CV*) (to determine

TABLE 1. Reliability codes for otolith readers.

Code	Definition
1 – Poor	Very difficult to interpret growth zones; annuli are vague and not always visible or have many checks; age is guessed.
2 – Fair	Growth zones are not well-defined and with many checks, but with identifiable and distinct growth patterns which allows the readers to estimate an age with a fair degree of confidence.
3 – Good	Growth zones are distinguished easily and in general, there is good definition between zones; a check is easily interpreted as a check and the reader has a good degree of confidence in the age estimate; this is a routine ageing reliability code.
4 – Excellent	A very clear otolith with distinctive growth zones, the reader would almost certainly give the same age when re-reading the otolith.

precision). *CV* is the ratio of standard deviation over the mean:

$$CVj = \frac{100\% \times +\sqrt{\sum (X_{ij} - X_j)^2 / R - 1}}{X_1}$$

where CV_j is the age precision estimated for the *j*th fish, X_{ij} is the ith age estimated of the *i*th fish, X_j is the mean age estimated of the *j*th fish, *R* is the number of times each fish is aged. The *CV* is preferred over other measures of precision because it takes into account the number of age groups in the population (longevity of the species), as it depends on an average age of the species (Campana, 2001).

Age bias plots measure the amount of bias between a set of age readings (Campana *et al*., 1995). Bias refers to systematic error between two age readings, for example if age readings are different at a certain range of ages (if one reader assigns older ages to all old fish than the other reader does). They are the most widely used for comparison of ages presently employed because it is visually easy to interpret. The age bias plot itself is an age-by-age measure of deviation away from a reference value (1:1 age determination) and it can clearly show over- or under-estimation even if ageing error is restricted to the youngest or oldest fish (Campana, 2001) (Fig. 6). It is important to note that in the example shown in Fig. 6A, there is no bias and seemingly high precision, but the ages may not be accurate. Only validation studies can give an indication of accuracy. The age bias plot example in Fig. 6B, however, shows extreme bias at older ages.

Accuracy is determined by validating age methodology. At NAFC, both the whole and thin-sectioned otolith techniques have been validated using a number of techniques (Dwyer *et al.*, 2003). Precision, on the other hand, refers to the closeness of two or more age readings. Precision should be as high as possible (i.e. variation should be low). It is noted that institutions that carry out age reading periodically check age readers for an acceptable level of precision (the actual level depends on the species, how old the stock gets, how long a reader has been ageing the species and whether the species has been aged for a long period of time). The reference collection can be used to carry out cross-checks between readers. Occasionally exchanges of otoliths are done among institutions. There was also a yellowtail flounder age reading workshop held in 2001, which provided an opportunity for scientists to discuss such issues and make the recommendation that a number of quality control tests to be carried out (Walsh and Burnett, 2002). However, it has taken a long time for a number of these recommendations to be implemented. Based on the recommendations from the workshop, there has been one recent attempt to exchange otoliths and age data between institutes (e.g. between the St. Andrews Biological Station, DFO New Brunswick, the National Marine Fisheries Service, NOAA, Woods Hole, Massachusetts., and the NAFC); however precision between these institutes remains a difficulty as they use different ageing methodology for yellowtail flounder. While most institutions have quality control programs in place, progress at NAFC has been relatively slow; however the reading of yellowtail flounder otoliths using the thin-sectioning method is relatively new, and therefore quality control programs are just beginning.

Using otoliths to age fish in general requires special training from a person knowledgeable about the otolith structure and the unique growth patterns of each species. Where possible, training is done with the aid of the reference collection. Usually a senior experienced ager shows the trainee

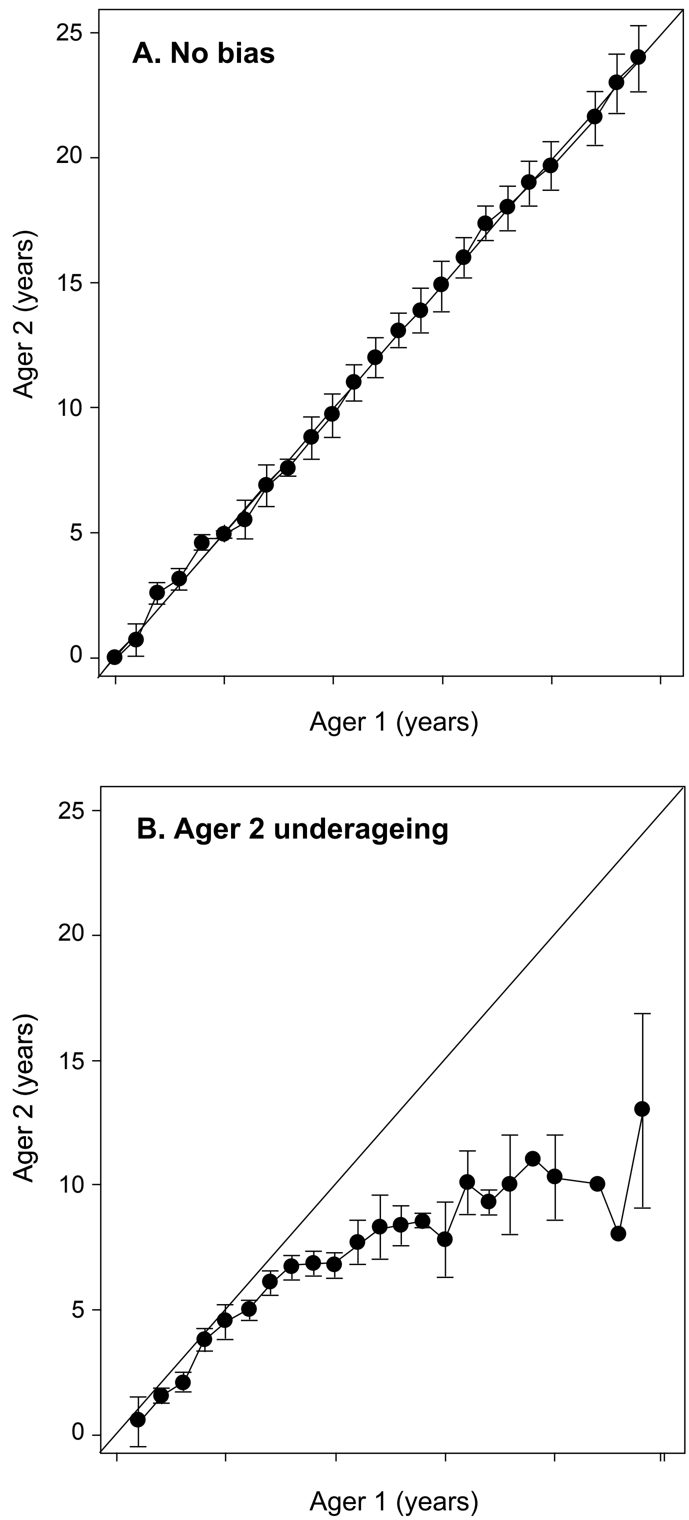


Fig. 6. Age bias plot examples, panel (**A**) showing no bias between the readers, and (**B**) showing extreme bias, with Age Reader 2 underageing, compared to Age Reader 1.

typical otolith growth patterns. The trainee is then introduced to developing an image search pattern to find out where the annuli should be located based on what is known about growth patterns. This is done by using a small number of otoliths (approximately 100). When adequate experience is acquired, the senior ager gives the trainee a number of otoliths (a small number at first, to compare, and then a larger number, e.g. upwards of 1 000 otoliths) to age independently. Usually these ages of the otoliths are then tested against the senior ager's readings. It is preferable that this is repeated to ensure the methods are understood adequately.

In addition, there should be no indication of bias in age bias plots between the senior reader and the trainee. Bias should be negligible in general, a rule-of-thumb is that bias as measured with an age bias plot not be present for more than ±0.75 years >3 years consecutively (see Fig. 6) and the coefficient of variation (*CV*) should be between 5–7%. The senior ager may choose to pick a number of these otoliths to repeat the process and improve on precision. The number of repeats depends on how the comparative readings proceed, particularly if there are a number of disagreements. If needed, the senior ager and trainee will re-age otoliths several times, and an acceptable level of precision must be reached before the trainee can go on to conduct ageing independently. The trainee's readings should be checked against a reference collection frequently once "production ageing" begins. Production ageing is the term used to describe the mass ageing done on large numbers of otoliths to develop age databases for fish. This training process ensures quality control and precision and results in the essential quality of age input data that will go into assessments.

General Ageing

In yellowtail flounder, while the two otoliths of a given fish are thought to have an equal number of annuli, often fewer annuli can be counted from the right otolith. The common reason is that the edges of the right otoliths are more compressed due to its position in the head of the fish (as mentioned above) (Fig. 2 and 3). For this reason, although both otoliths should be examined, at NAFC it is standard to use the number of annuli counted from the left otolith.

Yellowtail flounder are currently aged at the NAFC using whole otoliths for fish up to 25 cm in length, and the thin-sectioning method is used for all fish >25 cm. Figures 7 and 8 show a thin-sectioned otolith and a whole otolith from the same fish. At fish sizes less than 25 cm there are usually no discrepancies in ages estimated between methods (Fig. 7 and 8). Figure 9, however, demonstrates the situation whereby the whole otolith method does not detect the slower, lateral growth that occurs in older fish.

An "annulus" is defined as a continuous translucent zone and is deposited during winter months and is assumed 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 10A shows an otolith from a younger fish, with wide opaque zones which include checks, whereas Fig. 10B and C show an otolith section for an older fish with much narrower opaque zones.

One of the most important aspects of age reading otoliths (or any hardpart for that matter) is recognizing the growth pattern of the species. In cases where there are questionable checks or

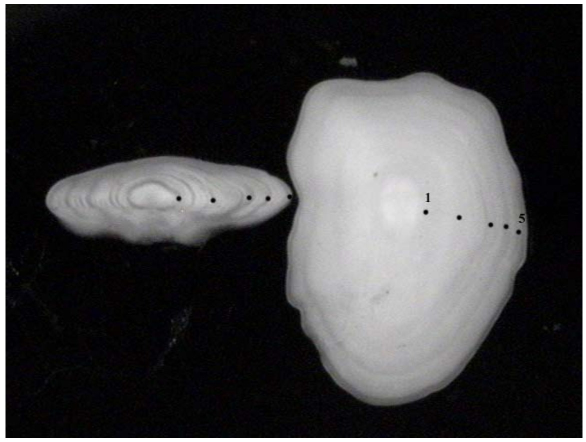


Fig. 7. Comparison of the thin-sectioned and whole otolith from the same fish, a 20 cm male yellowtail flounder. Both 'hardparts' are assigned an age of 5 years. Annuli are indicated by dots and numbers indicate the age. Fish was captured in October/November 1998.

cloudy areas on the otolith, the growth pattern recognition of a good reader will usually enable a reader to assign an age for that fish.

Axes to Read

When examining thin-sectioned otoliths, there are certain planes, or axes, along which reading can be carried out. Figure 11 shows a diagram of a thin-sectioned otolith, and the axes are described in the following text. This can be useful when discussing an assigned age with other readers of a particular otolith. Axis I is a straight line from the nucleus to either the dorsal or ventral tips. Axis II is closer to the *sulcus* about midway between Axis I and the *sulcus*. Axis III is closer to the *sulcus* again. Fast growth tends to occur along the dorsal and ventral tips of the otoliths and therefore Axis I is commonly used to age young fish. There are a lot of white opaque areas on this axis, because of the wide growth zones during the younger years. Axis II is closer to the *sulcus* and the annuli are more compressed. Axis III is the closest to the *sulcus* and is the most compressed. Otoliths from older fish, in which growth has slowed, should be read along Axes II and III. Axis I is excellent for reading the annuli of young fish otoliths, as the growth zones are clear. However the annuli themselves tend to appear "stretched out" and this makes checks appear prominent. Therefore they may be misinterpreted as annuli. Alternatively for older fish, Axes II and III could be used for ageing; however the disadvantage of these axes is that the annuli are so narrow that checks may also be misinterpreted as annuli. Readers should try and follow annuli they see in Axis I back to the *sulcus* area (Axis III) (Fig. 12).

It is more difficult to assign axes to whole otoliths, though the rostrum tends to grow faster than the rest of the otolith, and therefore growth zones appear more stretched out in this area.

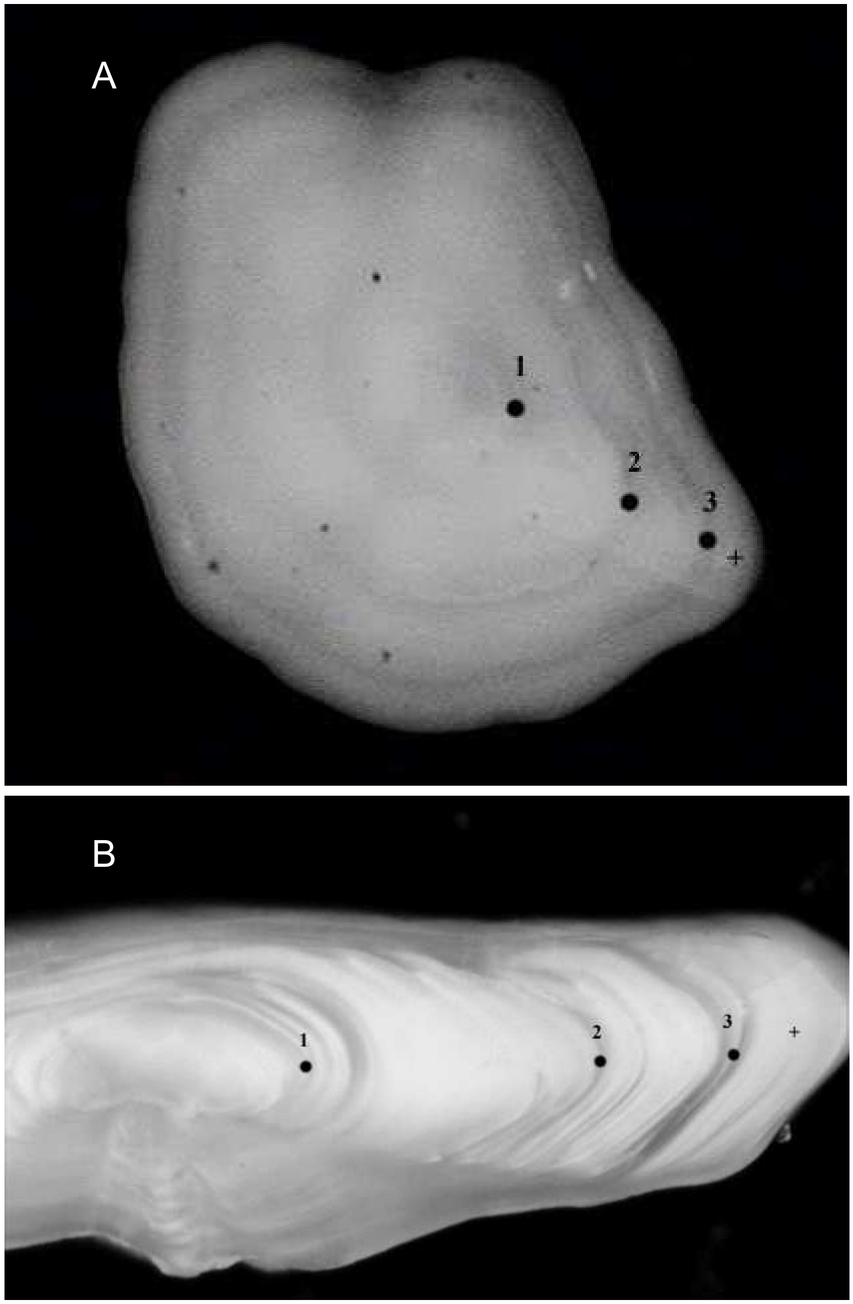


Fig. 8. Comparison of the same otolith, read whole (**A**) and sectioned (**B**). This otolith was taken from a 23 cm female yellowtail flounder and was aged as 3 years old. It was captured in November 1987. Annuli are indicated by dots and numbers indicate the age. Edge growth is also indicated by "+".

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

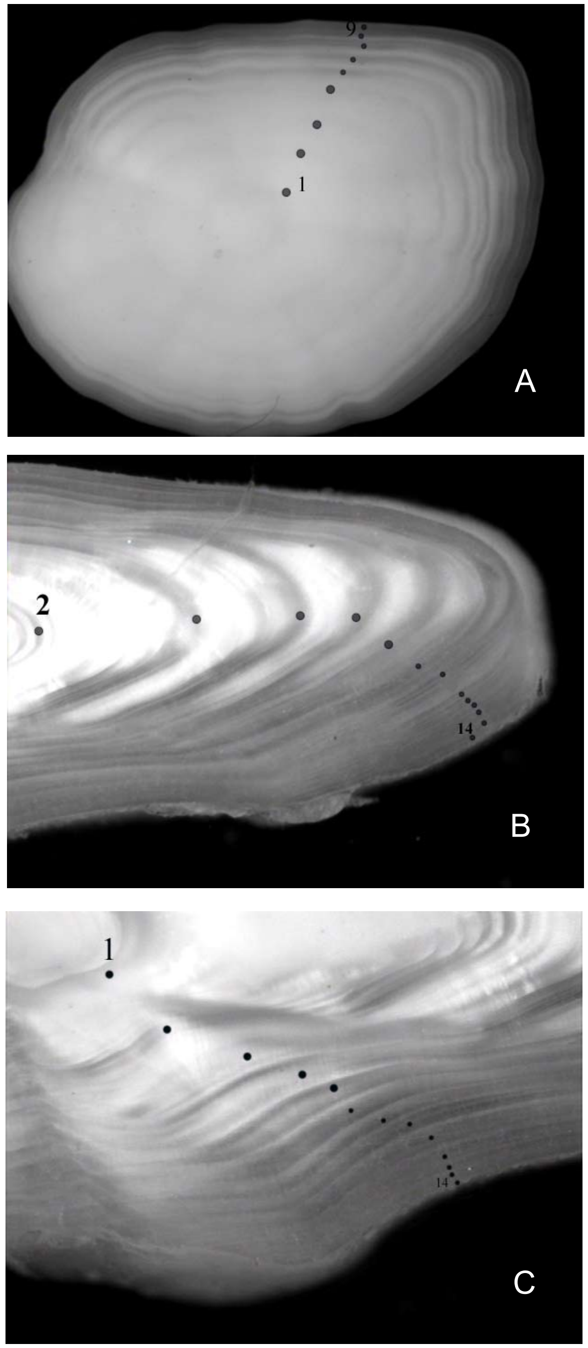


Fig. 9. Comparison between the whole (**A**) and sectioned (**B**) otolith from a 42 cm female yellowtail flounder captured in October/November 1998. At this size of fish, the whole otolith method fails and more annuli can be seen on the sectioned otolith (9 years old as compared to 14 years old). Fig. 10**C** shows a close-up of the sectioned otolith. Annuli are indicated by dots and numbers indicate the age.

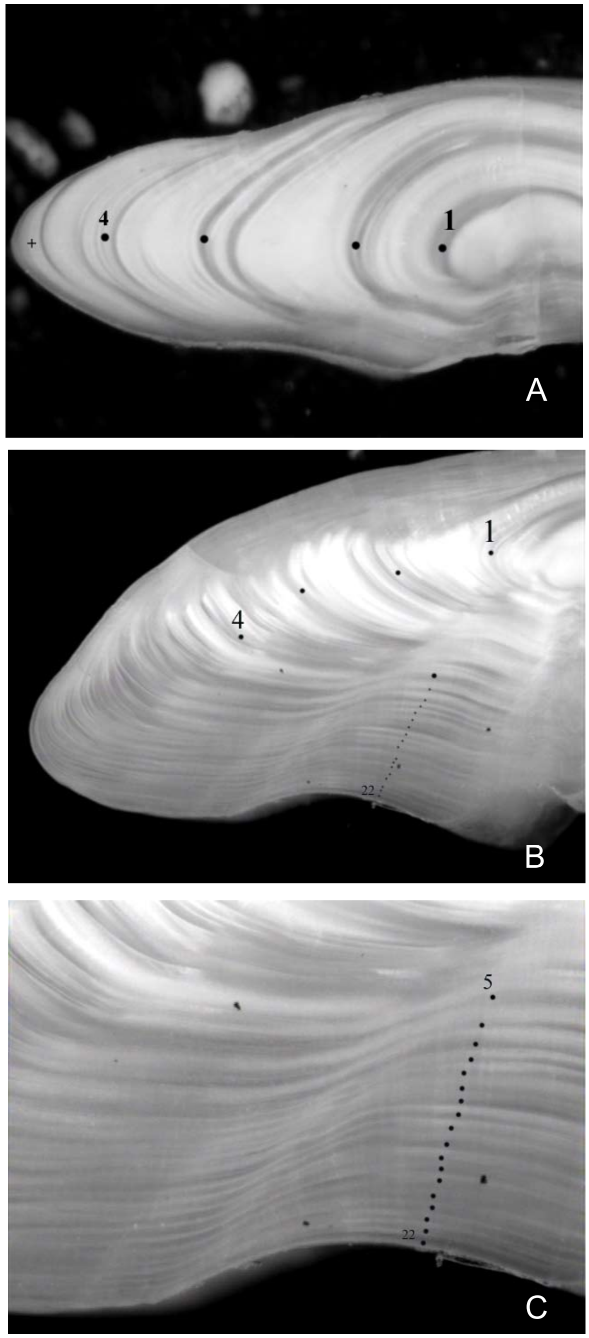


Fig. 10. Otoliths showing the difference in growth patterns between young fish (**A**) and old fish (**B** and **C**). Panel **A** shows an otolith from an 18 cm male yellowtail flounder, captured in November 2000, and Panel **B** shows an otolith from a 56 cm female, captured in August 1988. Panel **C** shows higher magnification of the outer annuli. Annuli are indicated by dots and numbers indicate the age.

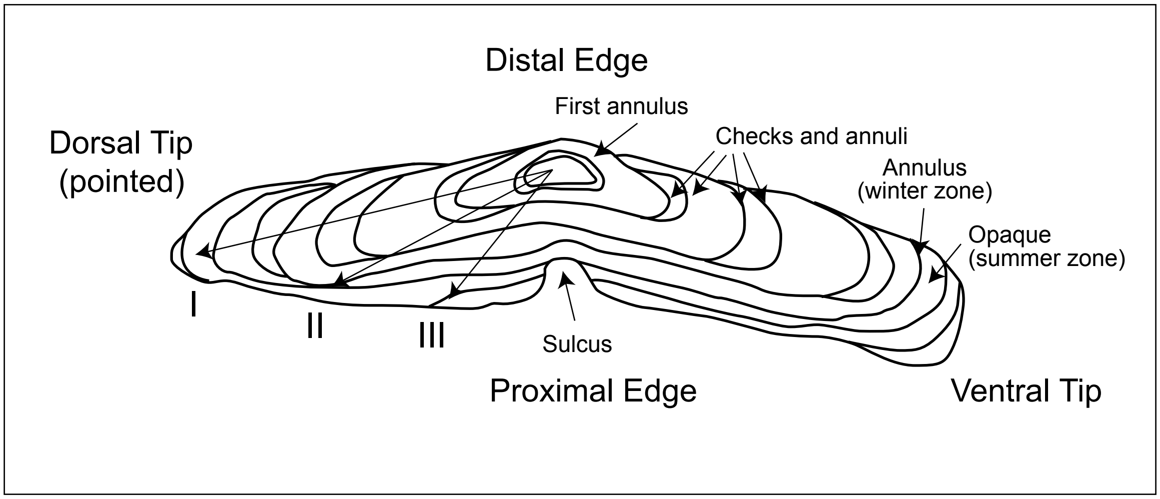


Fig. 11. Drawing of a yellowtail flounder otolith (modified from MacLellan, 1997) section showing typical ageing characteristics and areas used for counting annuli. Axis I indicates fast growth and Axes II and III indicate areas of slower growth. Any of the Axes can be used to age yellowtail flounder but often using Axis I and tracing it into Axes II and III is preferred. At older ages, Axis III is preferred for reading.

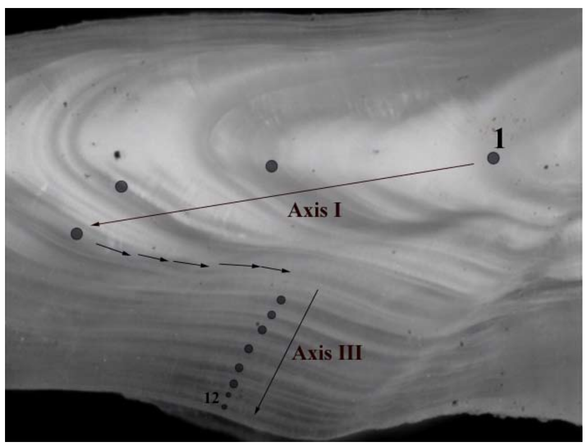


Fig. 12. Annotated image of a sectioned otolith from a 52 cm female yellowtail flounder, captured in May 1983, and aged as 12 years old. Annuli can be read along Axis I for the first years and then followed to the sulcus area (Axis III) where they are sometimes easier to see. Annuli are indicated by dots and numbers indicate the age.

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 yellowtail flounder, which hatched early in the summer, for example, will be picked up in the autumn surveys and a fish length mode related to this cohort will appear in length-frequency plots. Therefore, especially in younger fish, when growth is relatively fast, length-at-age will change over the year. At these ages length should not be used to estimate the age of a fish, however, length can sometimes be used to assist the reader after an age has been determined. See Fig. 13 and 14 for more details.

Variation in Size and Age

Age is related to length in yellowtail flounder in that age increases as length increases up to the point of sexual maturity. After sexual maturity, growth is slower and there is large variation in size and age across the length and size range of yellowtail flounder. Variation increases with age and length. Until about 25 cm, age does not usually vary more than ±1 year for 5 cm length groups. For example, all five otoliths in the photograph in Fig. 15 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 D in November. They were all captured in 1999–2000. They range in age from 4 to 5 years. However, Fig. 16 demonstrates fish that are the same size but are apparently older. These fish were captured in 1984–85, and in addition to variation in age-at-length it is possible that length-at-age has changed over the years.

Reading Whole Otoliths

In whole otoliths, the growth zones appear as alternating light and dark rings which are not particularly clear. The whole otolith is a flat and oval (though more round than otoliths from some other fish species) structure, with a groove running antero-posteriorally on the medial face of the otolith, and a slight rostrum (point) at the anterior end. The side opposite, which shows the *sulcus*,

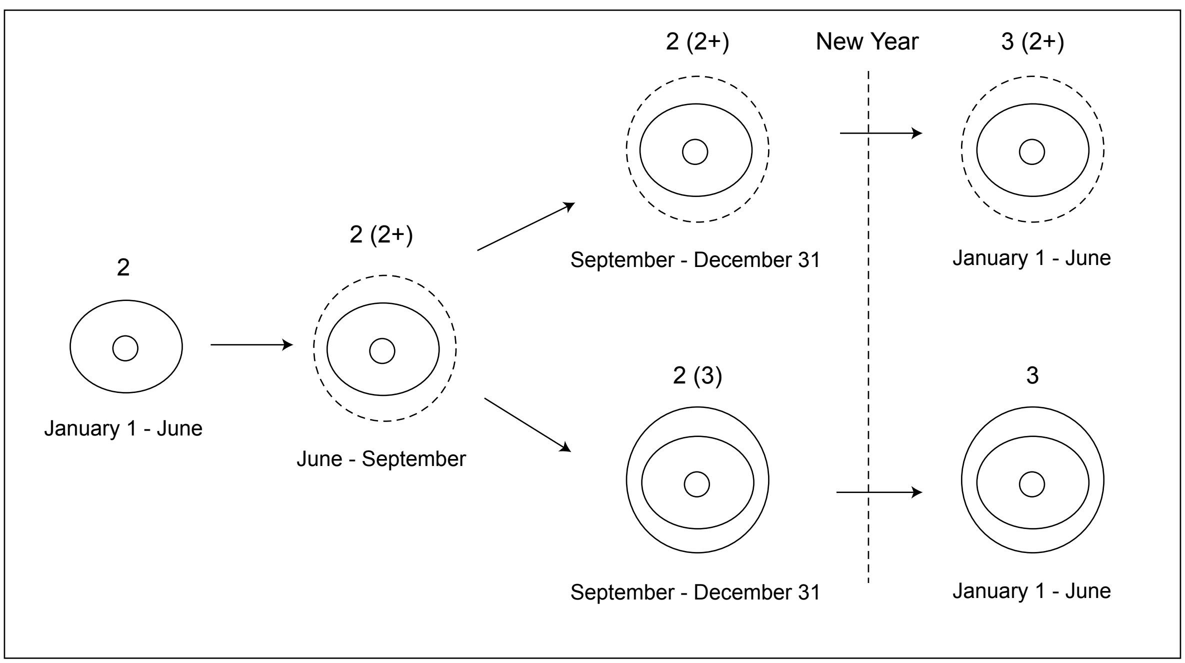


Fig. 13. Ageing according to the 1st January conventional birthdate, drawings represent an otolith showing growth taken place through the year. Solid lines represent annuli and dashed lines represent incomplete summer growth. The numbers above each represent the age that would be assigned to the fish based on the 1st January birthdate, while the number in brackets indicates the number of annuli actually seen (modified from MacLellan (1997)).

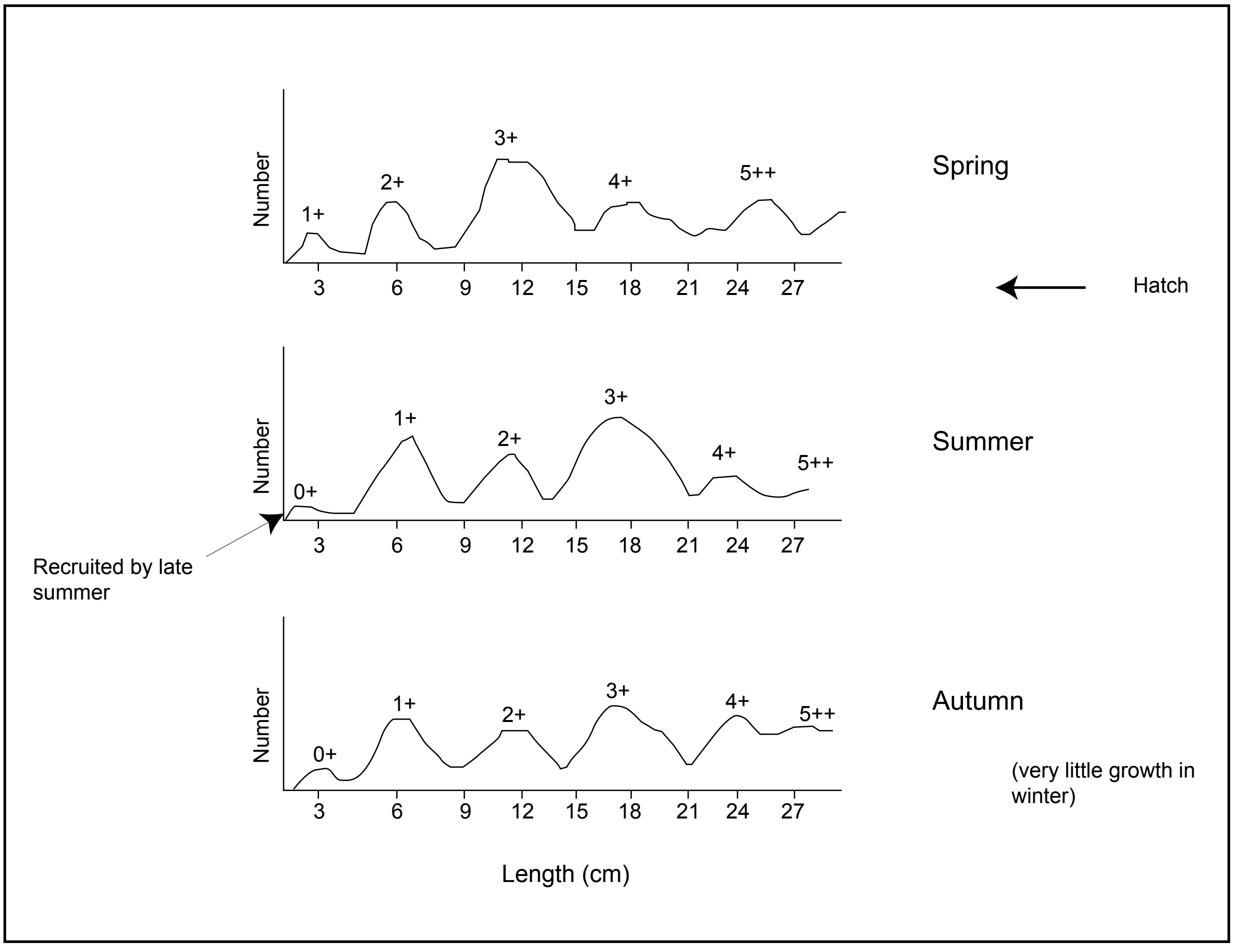


Fig. 14. A diagram showing a sample length-frequency plot and how modes change throughout the year, to demonstrate how using length-at-age for fish can be misleading because it changes over the year. "Hatch" indicates the point during the year yellowtail likely spawn, and when the larvae would hatch. They then may be recruited to the RV survey that autumn, as a mode representing the 0+ group.

is known as the lateral side. The otolith is read usually on the *sulcus* side (i.e. the lateral side) but in small fish can be read on either side of the otolith. Growth does not usually continue laterally and medially, but continues dorsally, underneath the perimeter of the otolith.

Whole otolith samples collected from yellowtail flounder are stored dry in envelopes and unlike in other institutes where ageing is done, they are not soaked in glycerin at NAFC. At the time of age determination, whole otoliths are simply removed from the envelope, placed in a black dish, covered with 95% ethyl alcohol (although water can be used). They are then examined under the microscope under reflected light at magnifications of about 7–25×. Whole otoliths are used for ageing yellowtail flounder up to 25 cm length.

Traditionally, the surface of whole otoliths was ground to remove the outer layers of calcium carbonate. This is done by placing the otolith on a specialized circular fast-moving stone mounted on a motor. This practice was believed to enhance the visibility of growth rings, but it has been found that there is no difference between the number of annuli read from ground *versus* non-ground otoliths (Dwyer *et al.*, 2001). However, if the reader feels it is necessary, there is no reason why

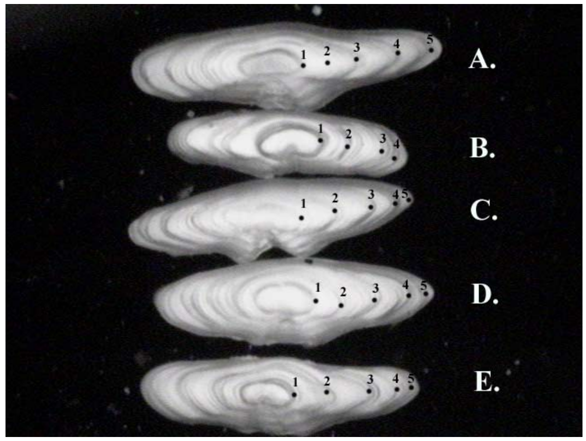


Fig. 15. All sections in this image have been taken from 25 cm fish, demonstrating only a small variation in age. They range in age from 4 to 5 years. Otoliths C and D were taken from males, while the rest were taken from females. All were captured in November 2000, except for otolith B, which was captured in October/ November 1999. Because of the time of year of capture, the otolith C would probably be aged as a 4-year old. Annuli are indicated by dots and numbers indicate the age.

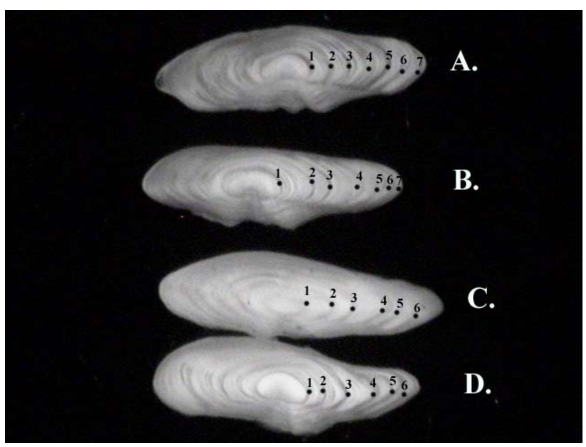


Fig. 16. Another comparison of otolith sections from four yellowtail flounder from 25 cm fish, with more variation in age. These fish were collected in 1984-1985 period and range in age from 6–7 years. Otoliths **A** (male) and **B** (female) were collected in August 1984; otoliths **C** (female) and **D** (male) were collected in January 1985. Annuli are indicated by dots and numbers indicate the age.

the otolith should not be ground. The only constraint is if the otolith is to be used for sectioning later, it should not be ground as this removes some of the ageing material from the sections. Since the current practice at NAFC is to section otoliths of yellowtail flounder >25 cm, surface grinding is not necessary to read them.

The distal surface of the left otolith is the side of the otolith most usually read. The proximal side contains the *sulcus*, a groove which runs down the length of the otolith and can obscure growth zones (Fig. 2). 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, otoliths from very young fish (0–4 years) tend to be fairly easy to read. In the older yellowtail flounder, the whole otoliths are not clear (annuli are not easily visible), although on occasion, some otoliths are found with very clear annuli, particularly in inshore yellowtail flounder (Fig. 17).

Annuli can be read along any growth axis, though growth zones may be clearer along the rostrum (Fig. 2). However, it is best to examine the sides of the otolith that correspond to the section, in order to obtain an edge type (see Section 8 on Edge Type) that is directly comparable with the thin-sectioned otoliths. The entire periphery of the otolith should be examined. When reading, each annulus should be followed around the complete otolith to make sure that it is an actual annulus,

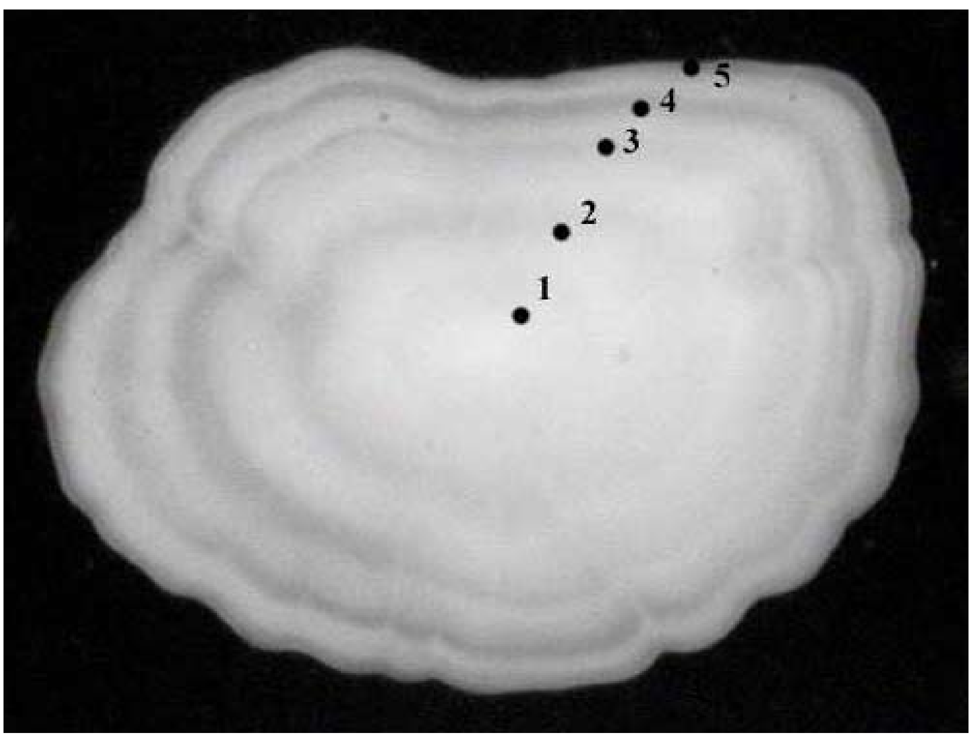


Fig. 17. A whole otolith from yellowtail flounder collected inshore in April 1999, from Witless Bay, Newfoundland. It is from a 5-year-old, 21 cm female fish. Annuli are indicated by dots and numbers indicate the age.

and not a check. Often, the rostrum (see Fig. 2) is the fastest growing area of the otolith and shows a different amount of new growth than the rest of the otolith. When determining age, it helps to examine a number of different growth axes, but it is best to pick one for determining the edge to maintain consistency. The age reader should attempt to manipulate the light and angle of the otolith being examined, to ensure the greatest clarity of the growth pattern is obtained.

In the characterization of whole otoliths, the term "crystallized" is used to refer to otoliths composed of vatarite, a structural variant of aragonite (Forsberg, 2001). It is unknown why crystallization occurs and may arise in one or both otoliths, or even in part of the otolith. For yellowtail flounder, fully crystallized otoliths should not be read. If they are partially crystallized, the otolith may be read, depending on the amount of crystallization. Figure 18 shows two examples of crystallized otoliths. Both are shinier than a regular otolith, but some crystallized otoliths are easier to read (Fig. 18A) than others (Fig. 18B).

As with thin-sections, whole otoliths may be difficult to read; thus 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 19 shows a drawing of this situation. The photograph of the otolith in Fig. 20 demonstrates this.

Preparation for Thin-Sectioning Otoliths

Thin-sectioning at NAFC described below follows the method outlined in Penttila and Dery, 1988, p. 9. Thin-sectioning of an otolith is a process that removes a thin portion of the otolith, usually across a specific plane, in this case, the dorso-ventral plane. It is a section made through the nucleus, using a low-speed saw with two wafer-thin blades. The purpose of thin-sectioning is to assist the reader to see more of the growth of the otolith, especially at older ages along Axis III when growth has slowed.

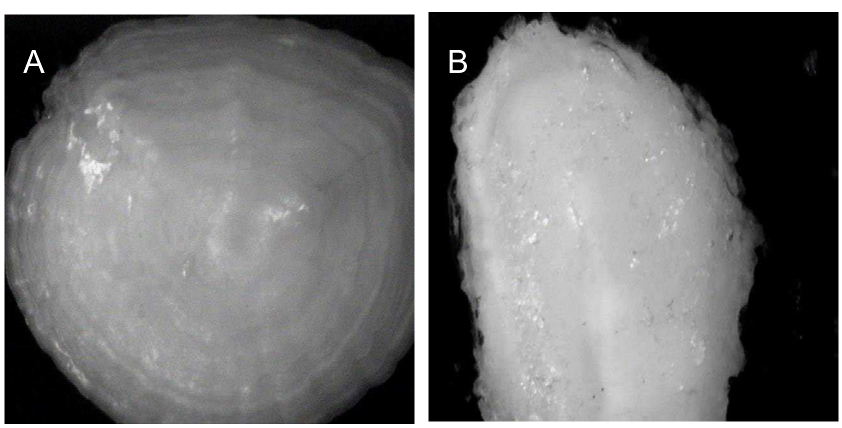


Fig. 18. A crystallized otolith from a 48 cm female yellowtail flounder (**A**) captured in August 1998. (**B**) shows a crystallized right otolith from a 46 cm female yellowtail flounder, captured in October/November 1999. It is possible that the otolith in (**A**) could be read, but the otolith photographed in (**B**) cannot be read.

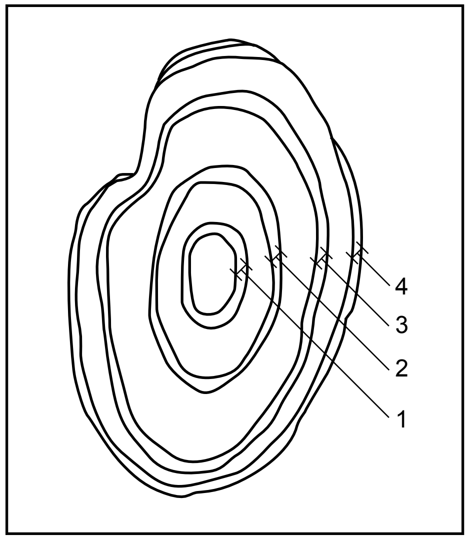


Fig. 19. Diagram of a whole otolith showing annuli present in a "lumped" pattern (Modified from Forsberg (2001)).

Thin-sectioning is performed on otoliths using a low-speed saw with double blades. Usually a section which is approximately 0.4 mm thick is cut and removed for ageing.

Supplies Required:

Low-speed saw
Diamond-tipped blades
Dressing stones
Paraffin Wax
Marking tags
Carbon, decolorizing
Calcium oxide
Double sided tape
Hot plate

Steps Employed to Thin-section Otoliths

Step 1: *Preparation of the Embedding Mixture*

The embedding mixture consists of approximately 4 parts melted wax, 3 parts calcium oxide and 1 part carbon. The wax is first melted in a container on the hot plate, and the calcium oxide and carbon are added to the wax and gently stirred in. The temperature should be controlled to ensure this mixture is not too runny or too thick. The amount of carbon added can be varied somewhat as the mixture is cleaner if the carbon is not used, however in the clearer wax mixture the otolith is harder to read.

Step 2: *Embedding the Otolith*

This step requires special care to orient the otolith correctly and that each otolith is properly labelled. Each otolith should be embedded in the wax mixture but has to be done in such a way that

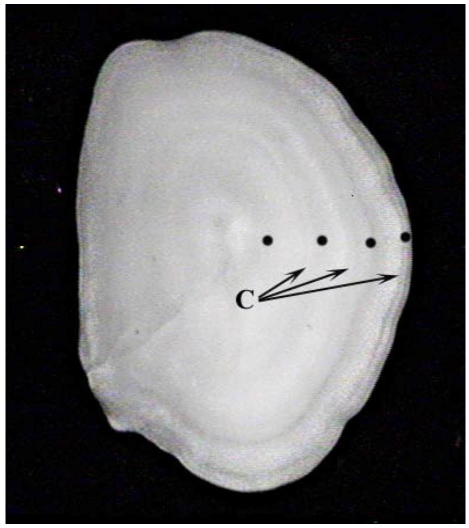


Fig. 20. A photograph showing an otolith where one would "lump" the checks and annuli. Counted this way, the fish would be aged as 4-years old. If the checks and annuli are split and all apparent "annuli" are counted, of the assigned age would be 7-years, which is likely inappropriate, based on knowledge of growth patterns. 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).

the nucleus lines up with the blades properly. A paper labelling tag is used for this purpose (see Fig. 21). Because the otolith cannot be seen once it has been embedded on the tag in the wax, one must ensure it is lined up properly first. To aid in orienting the otolith with the blades, a cross is pencilled in on the tag. A piece of double-sided tape is placed on the tag, which ensures the wax mixture stays attached to the tag, and then a drop of embedding mixture is dropped onto the cross marked on the tag. The otolith is then immediately but carefully oriented into the mixture, such that the *sulcus* is placed along the shorter line, with the nucleus lined up with the centre (Fig. 21).

The wax is then spread over the otolith, slightly more than just enough to cover the otolith. This wax mixture with the embedded otolith is usually a round spot, about the size of a thumbnail. A number indicating the sample being sectioned can be printed on the tag. The wax begins to harden almost immediately, but should be cool to the touch before sectioning.

The section cut out of the otolith is a dorso-ventral section. This step is very important. If the otolith is not oriented correctly, the saw will not cut through the nucleus and this affects the accuracy of the age reading.

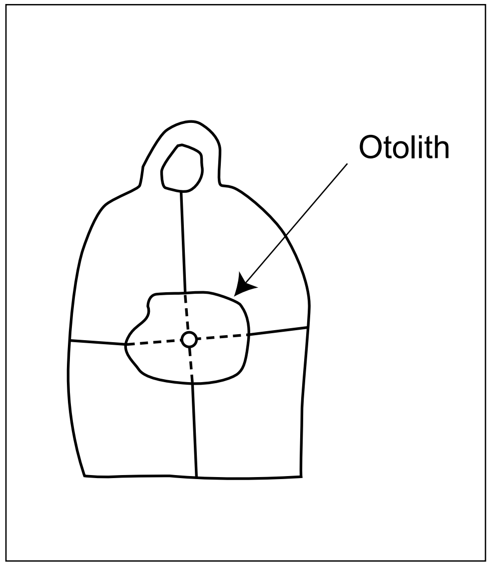


Fig. 21. Diagram of the otolith embedding position on a tag, to ensure that the saw removes a dorso-ventral section through the nucleus.

Step 3: ***Machine and Blade Maintenance***

Sections are cut using an Isomet low-speed saw. This saw contains a holding arm (the chuck) in which the tag (with the embedded otolith) is mounted. The section is removed by using two wafer-thin diamond-tipped blades to cut through the otolith. Particular attention must be paid to not damage the cutting edge of the blades.

The tray placed under the blades should be kept filled with cool distilled water (tap water should not be used as the tray is made of aluminum and corrodes easily). The purpose of the tray with water is to ensure the blades run through the water to keep them cool. A few drops of detergent should be added to the distilled water to keep the blades clean.

The machine permits cutting sections of various thickness. The thickness of the section is determined by the size of a spacer placed between the two wafer-thin blades. The operator of the saw and the objectives of the sectioning process control the blade spacing. There are a number of spacers that can be used. An old discarded blade can be used to fit between the blades, by making the diameter of the spacer slightly less than the diameter of the blades. Thin plastic pieces or cut Mylar can also be used as spacers. For regular ageing of yellowtail flounder otoliths, spacing of about 0.3–0.5 mm is appropriate. Calipers should be used to measure the section and ensure it is the suitable thickness. Sections cut less thin than 0.3 mm tend to get broken and lost easily.

Step 4: ***Placing the Wax Block on the Machine***

The wax block with the embedded otolith on the tag must be placed in the chuck of the machine very carefully. The most important feature is to orient it correctly such that the lengthwise cross pencil mark lines up between the two blades and the shorter line is precisely parallel to the blades. This orientation is to make sure the saw is sectioning the block correctly. In addition, the angle of the tag to the saw is important and the dorso-ventral edge of the embedded otolith should be placed perpendicular to the blades themselves. The saw should be started and the blades spinning slowly before the chuck and saw arm is laid on the blades. If the chuck is laid on the blades before the blades are spinning, the blades may become warped.

The block is now mounted and once it is firmly in place on the chuck, it is ready for sectioning. The chuck is lowered onto the slowly rotating blades. The sectioning should begin with gentle movements at the start. This is done by holding the chuck above the blades, and slowly beginning the rotation of blades from the mid-cycle position. Then slowly and gently the chuck should be lowered such that the wax block with the otolith contacts the blades. The blade rotation should then be turned up to maximum as the sectioning process begins. As the chuck lowers further, two blades cut the section out of the otolith.

Step 5: ***Collecting the Sectioned Materials***

The machine can be set so that the automatic shut-off switch will stop the blade rotation when it has completed the sectioning of the otolith. The wax block with the tag should then be removed from the chuck and the thin-section taken out.

The sectioned otoliths should be collected and placed between the folded piece of paper. A small piece of dark construction paper can be placed at the bottom of this fold so that the otolith is easier to see. This folded paper can then be placed, along with the two remaining pieces of the otolith in an envelope. This is not an ideal storage system, as the section is loose in the paper fold and can be lost easily; this is one drawback of this system and is part of the reason other methods are being examined.

Step 6: ***Care of the Blades after Sectioning***

The blades should be cleaned daily by washing gently with hot water and detergent and then rinsing with distilled water. The blades should not be left mounted on the saw overnight. When not in use, the blades should be laid flat on a surface that is safely away from the work space.

After a prolonged period of use, the blades will need to be dressed. This period is subjectively determined by the amount of usage the blades get. The more frequently they are used, the more frequently they should be dressed. If used consistently, a good rule of thumb is to dress the blades every 3–5 days, however this is mostly based on personal judgment.

There are special dressing stones for the Norton blades. To dress the blade, the dressing stone should first be fitted tightly on a special chuck on the Isomet low-speed saw. The blade is then mounted in its sectioning position on the saw. Dressing begins by first turning the machine on to a slow blade rotation. Then as the blade is spinning slowly the dressing stone in the chuck is lowered down onto the blade. Allow the blade to run through about ¼ cm on the dressing stone, but not past the diamond tipped edge of the blade. This should be repeated about 5–7 times for each blade. Blades should be run through one at a time (not double).

Regarding thin-sectioning otoliths of fish less than 25 cm: usually these otoliths are not sectioned (they are read using a whole otolith), but if the ager wishes to do so, the otolith should be cut in half, using only one blade, and the two pieces of otolith can be used to obtain an age. The double saw method (above) is difficult to use, as the otolith is too small and often gets crushed or easily lost.

Whole Otoliths *versus* Thin-Sectioned Otoliths

For the first 4–5 years of a yellowtail flounder, whole otoliths and thin-sectioned otoliths can be compared directly and one can see the slowing of growth in the whole otolith as fish get older. The main difference is that one cannot distinguish individual growth zones in older fish when reading whole otoliths. When whole otoliths and sections are directly compared, it can be noted that whole otoliths have fewer visible checks than seen in the sections but the annuli on the edge of the otolith are harder to see, especially in older fish (>6–8 years) (see Fig. 22–26). Figures 22–26 show a comparison between some whole and sectioned otoliths. For fish 25 cm or less, the comparison in annuli between whole and thin-sectioned otoliths is fairly straightforward and most of the features seen in the thin-section can be seen on the surface of the whole otolith, including checks (Fig. 22 and 23). In Fig. 24, a comparison of edge types is similar in both the whole and sectioned otolith. Figure 25 shows how the actual placement of the section is important, as the whole otolith shows that the last annulus has not fully formed around the perimeter of the otolith. If sectioned higher, this last annulus would not likely have been seen. Finally, in Fig. 26, it is shown that a 30 cm fish will show differences between the surface read otolith and the sectioned otolith. This difference is more pronounced with age.

Reading Thin-Sectioned Otoliths

Where possible, the left otolith is used for thin-sectioning but if not available, the right otolith can be sectioned, taking care to section directly through the nucleus. If the left otolith is available, it is beneficial to the age reader to have a thin-section of it and keep the right otolith whole for comparison. This left section is stored in a fold of paper and stored in an envelope.

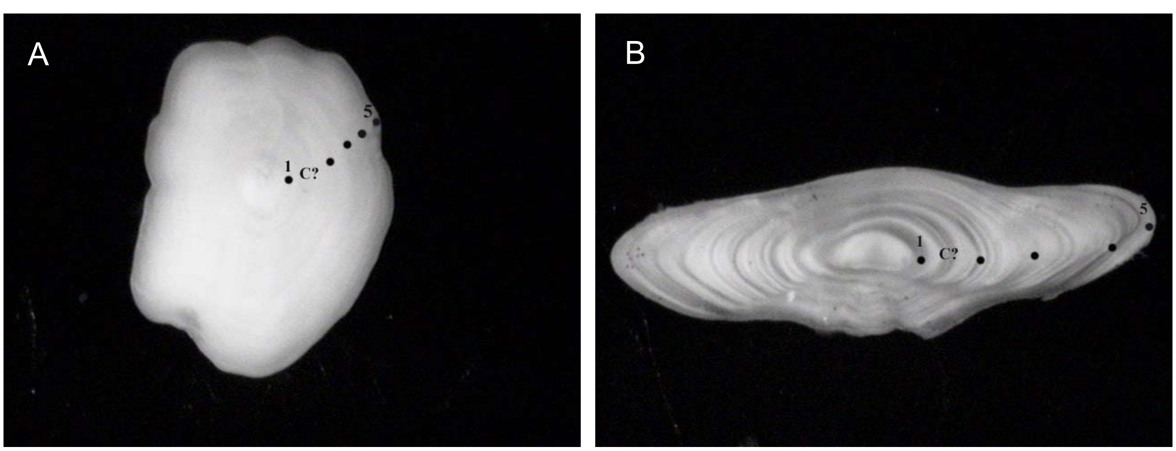


Fig. 22. Comparison of (**A**) the whole otolith to (**B**) the sectioned otolith. This otolith was taken from a 19 cm female captured in June 1998 and aged as 5-years. Annuli are indicated by dots and numbers indicate the age. In fish of this size, the same age is obtained from the reading of either the whole otolith and sectioned otolith, and the observed patterns of growth are the same.

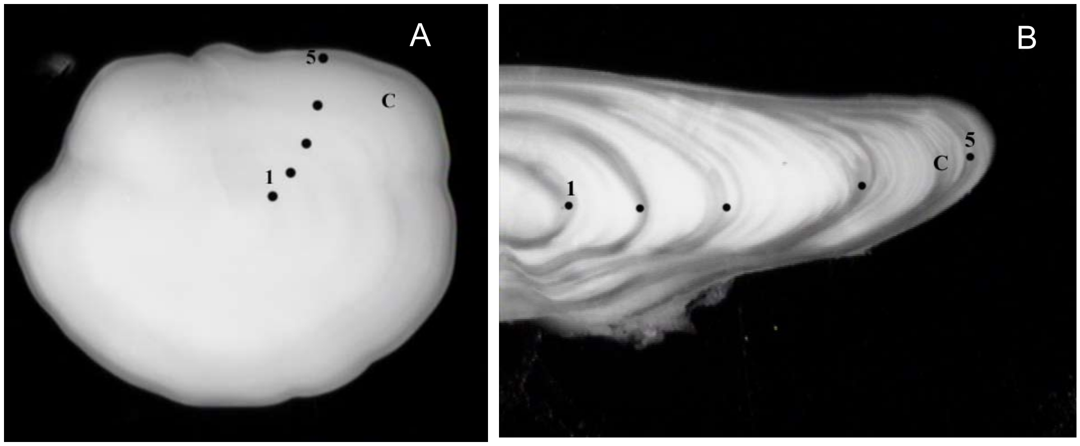


Fig. 23. Comparison of the whole otolith (**A**) to the sectioned otolith (**B**). This otolith was taken from a 23 cm female captured in June 1998 and aged as 5-years. Annuli are indicated by dots and numbers indicate the age. Notice again that the patterns of growth are the same on the whole and sectioned otolith. It is unusual that the first three zones of growth are slow, and the next two faster.

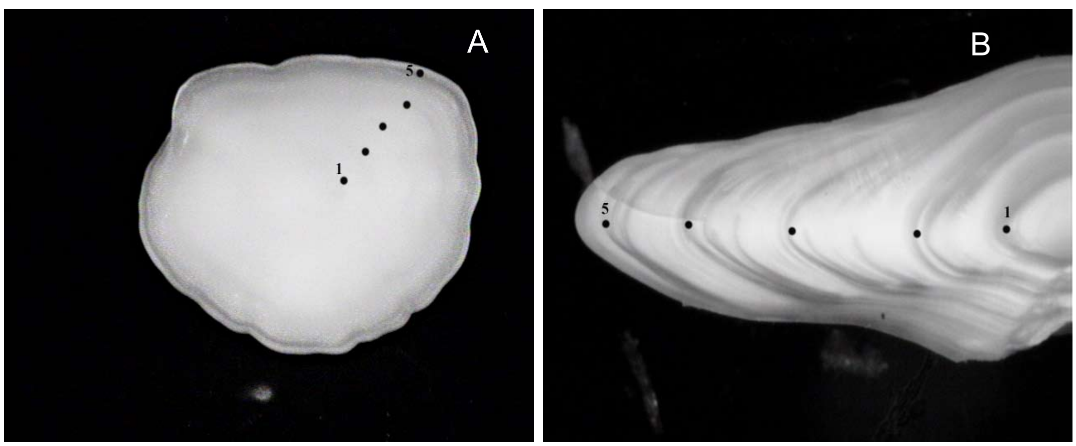


Fig. 24. Comparison of the whole otolith (**A**) to the sectioned otolith (**B**). This otolith was taken from a 25 cm female captured in June 1998 and was again aged as 5-years (as in Fig 23). Annuli are indicated by dots and numbers indicate the age.

Sections can be examined under either transmitted light or reflected light, but in most cases reflected light is adequate and it is best to be consistent. Ethyl alcohol is used to cover the section because it enhances the annuli under light. Observations are made under 25–40× magnification with a dissecting scope, but they should also be examined for checks and other unusual anomalies under lower power (7.5–20×).

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 (Fig. 4).

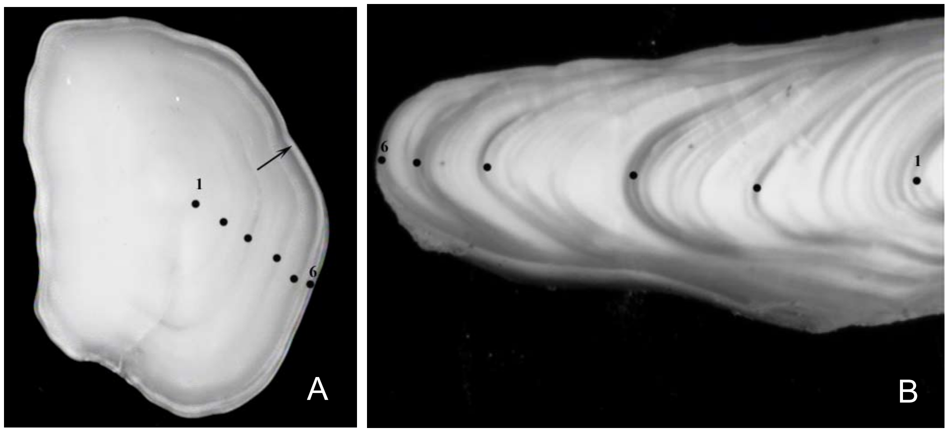
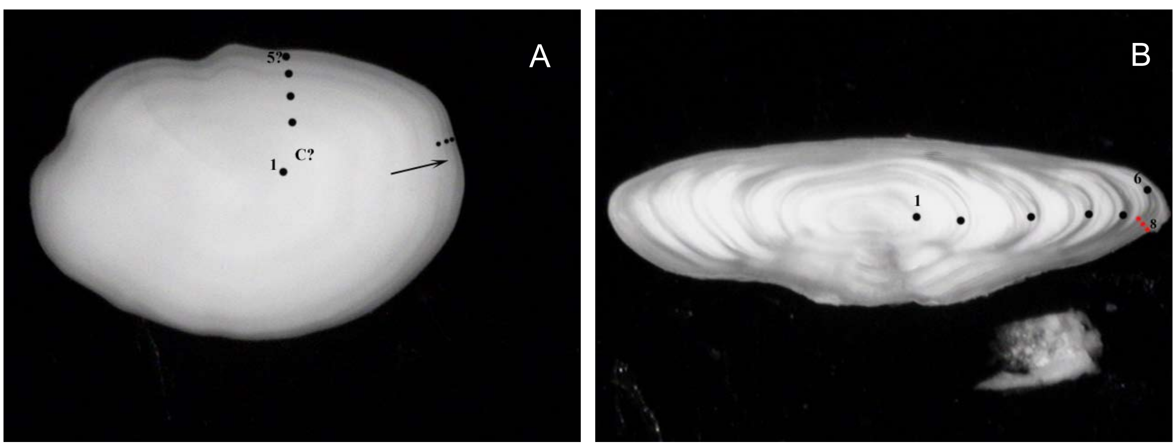


Fig. 25. Comparison of the whole otolith (**A**) to the sectioned otolith (**B**). This otolith was taken from a 28 cm male captured in June 1998 and was aged as 6-years. Annuli are indicated by dots and numbers indicate the age. The arrow points to an area on the otolith where the new annulus has not formed yet. If the otolith had been cut in a different area, it would have been difficult to see this new growth. This demonstrates that it is often important to examine both the whole otolith and the sectioned otolith.



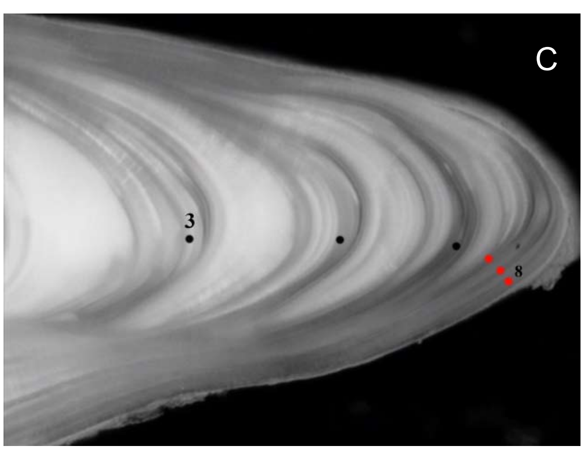


Fig. 26. Comparison of the whole otolith (**A**) to the sectioned otolith (**B** and **C**). This otolith was taken from a 30 cm female captured in August 1999. Annuli are indicated by dots and numbers indicate the age. This was aged as 5-years from the whole otolith although the arrow indicates that there may be more annuli present. However, it is only when it is sectioned that three more annuli can be seen 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 by readers.

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.

The following sections may be used as a guide for reading thin-sectioned otoliths.

Annuli *versus* Checks

Often, annuli and checks are difficult to distinguish. An annulus is defined as the combination of both the fast growth and slow growth periods of each year (one full summer and one full winter of growth). However, for age readers, an annulus is simply the ring (under reflected light, the dark ring) that is counted to indicate one year. It is usually considered to be an annulus if the ring is continuous (appears in all axes) around the otolith and the growth pattern fits with its location. Checks, comparatively, 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 diffused and in general, not consistent with the growth pattern. Checks are also called sub-annual markings or zones.

Similarly as with whole otoliths, annuli and subsequent opaque zones are large up to 5–7 years, after which, maturation occurs and there is a considerable narrowing of annuli. Knowledge of typical growth patterns helps to distinguish annuli from checks in the younger years. Checks appear more prominent under high magnification, and reducing the magnification tends to increase the ability to differentiate between annuli and checks. For example, in Fig. 27A at a magnification of about 30×, the potential check ("C") appears strong and continuous, although when it is traced to the *sulcus* it looks as if it joins with the annulus. Unless carefully traced, this check could easily be interpreted as an annulus and read as a growth zone. However, when examined under lower power (7.5×) as shown in Fig. 27B, it becomes apparent that this is a check. It is also known that annuli do not form so closely together in the younger years; thus using that knowledge may help determine if the ring is a check or an annulus. This example 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.

In addition to environmental stress, checks may also be caused by maturation, spawning or metamorphosis. Figure 28 shows an otolith from a fish which might display a "spawning check" ("SC"). This would occur at the time of first maturation, as it is an immense change in the physiology of the fish. For yellowtail flounder stocks in the Grand Bank area, length of 50% maturity ($L_{50\%}$) is believed to be about 25 cm in recent years for males and 32.5 cm in recent years for females (Walsh and Morgan, 1999).

In Fig. 29, 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.

Therefore, although annuli and checks may be difficult to distinguish, usually the knowledge of growth patterns and changing the magnification and light to follow the rings throughout the otolith will help the age reader reach a decision.

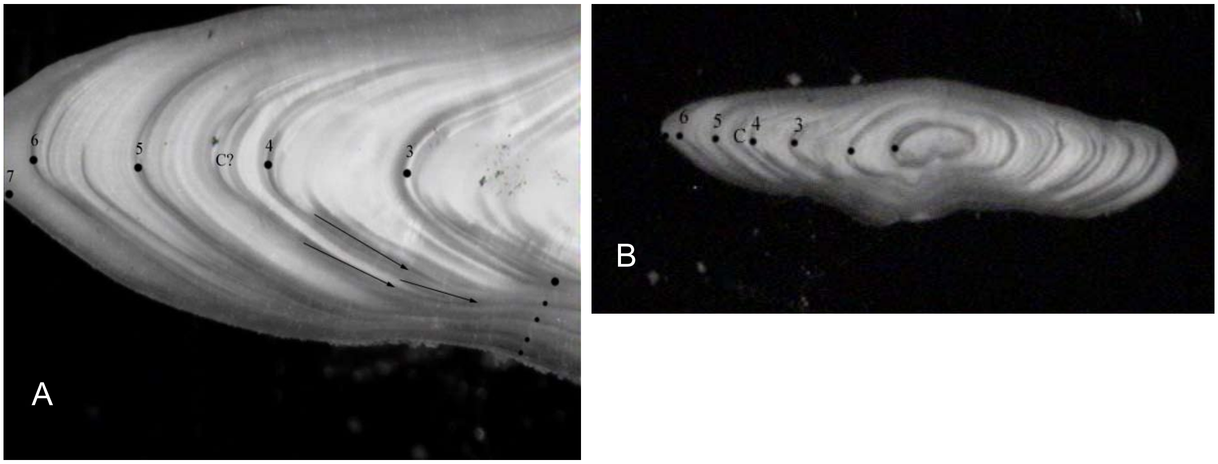


Fig. 27. Annotated photographs of the same otolith from a 32 cm female captured in November 1999, at high (**A**) and low (**B**) magnification. At high power, often a check ("C") will appear more prominent than it actually is, and should be examined under low power so that the questionable ring can be examined in relation to the other complete rings. In this case, knowledge of growth zones enables us to determine if the ring is actually a check. Annuli are indicated by dots and numbers indicate the age. Arrows are used to indicate places where annuli can be followed into the sulcal area.

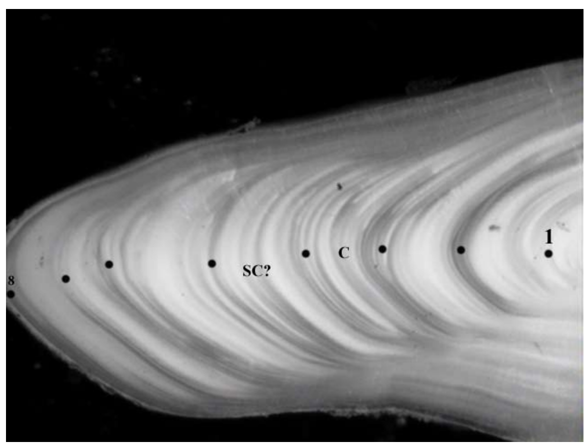


Fig. 28. Annotated photograph of a sectioned otolith from a 33 cm female captured in July 1999, showing an example of a check ("C") and possible spawning check ("SC") which cannot be traced into the sulcus area (discontinuous). The check between the 4th and 5th annuli is marked as a possibe spawning check. Annuli are indicated by dots and numbers indicate the age.

First Annulus and Difficulties with a "Second Annulus" (Problem Areas)

In yellowtail flounder, the first annulus can range in appearance from round to oval in shape in cross-section. The round appearance is more common. The first annular zone is typically small

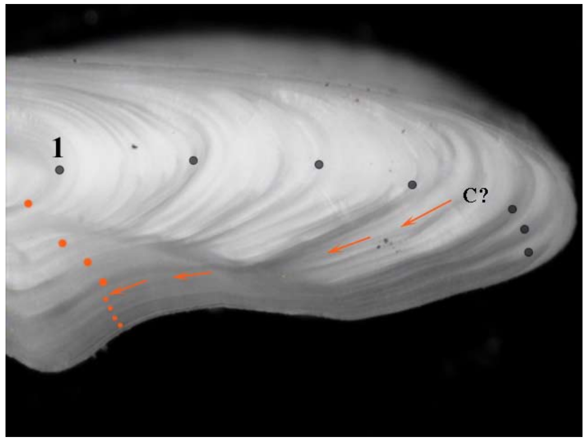


Fig. 29. An annotated photograph of a sectioned otolith from a 44 cm male captured in October 1981, showing an example of a potential check ("C"). Because the "check" can be traced back into the sulcus, it appears as if it is a true annulus. Annuli are indicated by dots and numbers indicate the age.

(on average 0.71 mm in diameter) but distinct, which helps to determine which translucent zone is the one to start counting from. However, it is important to note that there may be variation in the size of the first annulus, subject to date of spawning resulting from a prolonged spawning period. In cases where the fish spawns early in spring, the first year may show a lot of opaque growth and in cases where the fish spawns later in the summer, the first year may have little opaque growth. Sometimes the first annulus is unusually small or the distance between annuli 1 and 2 is smaller than between 2 and 3. This might be because the fish was spawned at the latter part of the spawning period (later in summer) and had a short first growing season (Fig. 30). A good example of this is seen in Pacific halibut (*Hippoglossus stenolepis*) (Forsberg, 2001).

Figure 31 shows a sectioned otolith from a 2-year-old yellowtail flounder that shows a typical growth pattern for the first two years. Figure 32 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.

In some cases, thin-sections reveal a strong check outside the first annulus, which may or may not be the actual second annulus (Fig. 33). It often appears to be a second annulus, but in fact it should probably 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 (Dwyer *et al.*, 2001). It should therefore be called a check and at the time of writing, at the NAFC it will be called a check. If the second apparent zone is strong and can be followed into the *sulcus*, it is counted as an annulus, but in this case some judgment should be used. Typically, the situation is such that

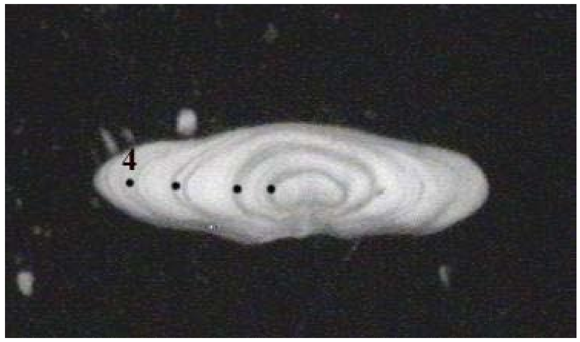


Fig. 30. An photograph of a sectioned otolith from a 4-year-old, 18 cm male captured in November 2000. The second ring would probably be interpreted as an annulus, because the spacing may indicate otolith spacing from a late-spawning fish. Annuli are indicated by dots and numbers indicate the age.

Fig. 31. A photograph of a whole otolith from a 2 year-old, 12 cm male captured in October/November 1999, showing typical growth patterns in young fish. Annuli are indicated by dots and numbers indicate the age.

the second annual zone is wide (Fig. 24B). However, in the "small second annulus" situation, the second zone looks very close to the first annulus (Fig. 33).

Growth before Maturation

After the first annulus, it is unusual for an annual zone to be less in width than the following one (next year's growth), but in some cases it does happen. Unlike the round first annulus (and sometimes second annulus), the third to fifth annuli tend to be more pointed and "stretched-out" along the longest growth axis. 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".

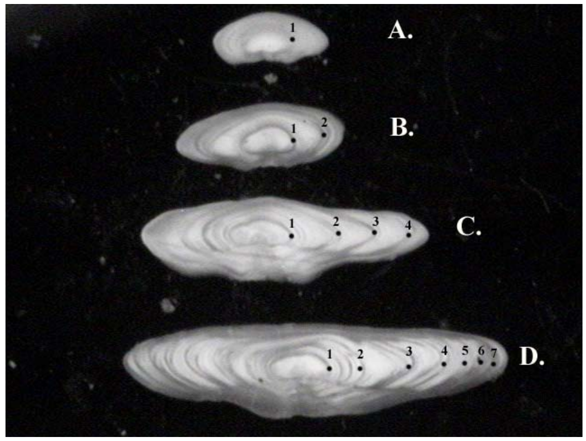


Fig. 32. A composite comparison of otolith sections from fish of different sizes. (**A**) is from a 6.5 cm female captured in November 2000, (**B**) is from a 12 cm male captured in October/November 1999, (**C**) is from a 23 cm female, and (**D**) is from a 39 cm female also captured in November 2000. Note the edges on the otolith sections. In (**D**) note that a new annulus has started to form but because it was captured in November it is not counted. Annuli are indicated by dots and numbers indicate the age.

Growth after Maturation (Older Fish)

After maturation, both somatic and otolith growth slow considerably. 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. 11). As fish get older, the otolith material grows laterally and becomes more difficult to see on the surface of an otolith. Figures 34–36 are examples of the lateral growth that continues in otoliths from older yellowtail flounder (>7–10 years). At high magnification, one can view the small growth increments that occur at older ages.

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 37 shows an example of this, which tends to be fairly common in yellowtail flounder. Note also a potential spawning check in Fig. 37.

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. 38A and B). Efforts should be made to recognize these patterns and ensure that both rings are not counted. Figure 39 shows an image of a "split" first annulus.

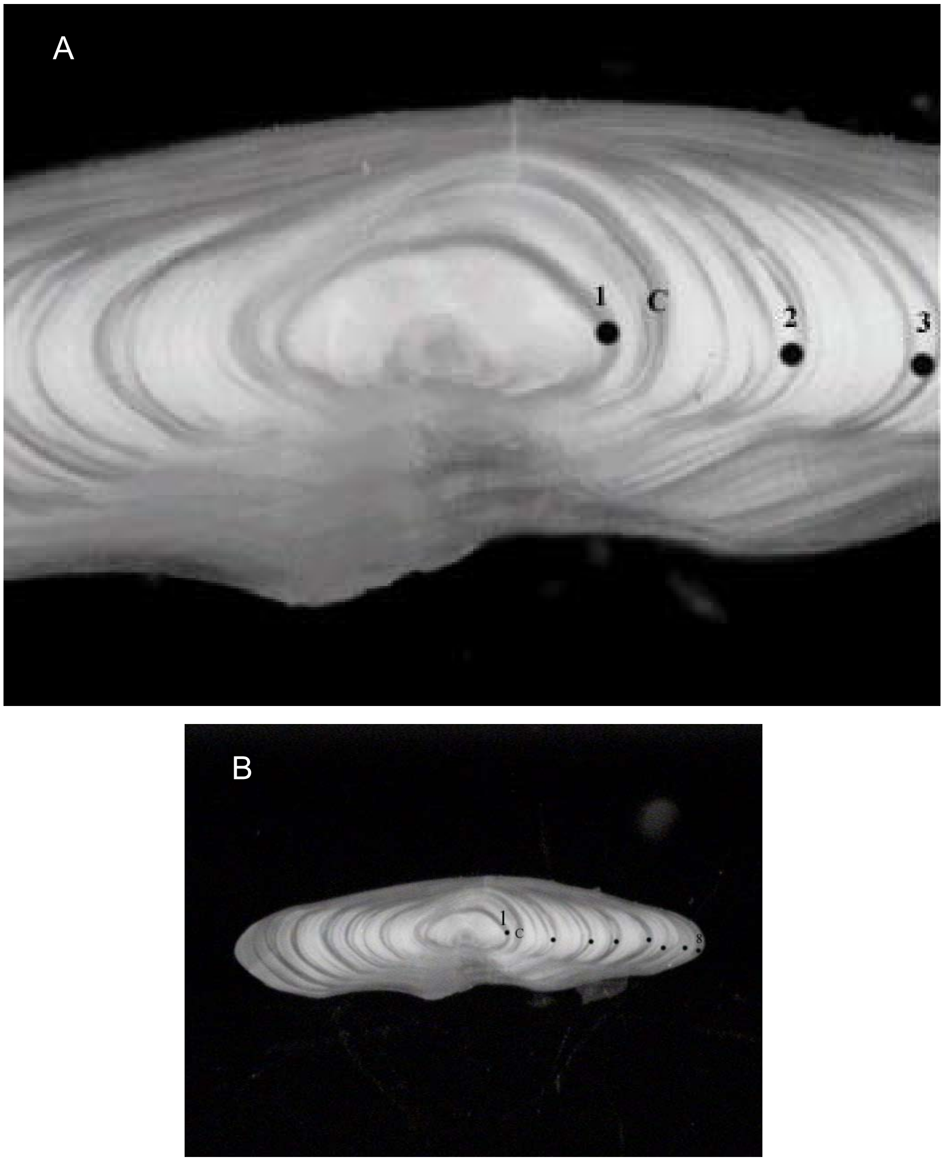


Fig. 33. An annotated photograph of a sectioned otolith from a 27 cm female captured in January 1985, taken at high (**A**) and low (**B**) magnification, showing an unlikely "second annulus" (indicated by "C"). It is more likely a strong check than a true annulus. Annuli are indicated by dots.

Annuli may be composed of numerous translucent zones or checks forming close together (called "checky") (see Fig. 40) 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.

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. 41.

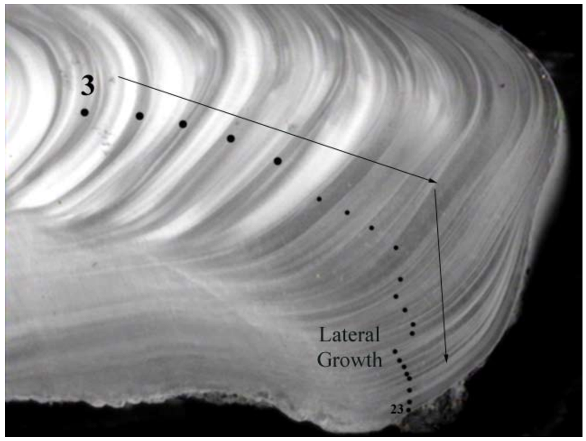


Fig. 34. An annotated photograph showing an example of the lateral growth that exists in otolith sections from older yellowtail flounder. This fish was recorded as a 33 cm female that was captured in July 1999. It is unexpected that a fish of this size would be 23 years old and it is believed there might have been a mistake in recording the length on the envelope label. Annuli are indicated by dots and numbers indicate the age.

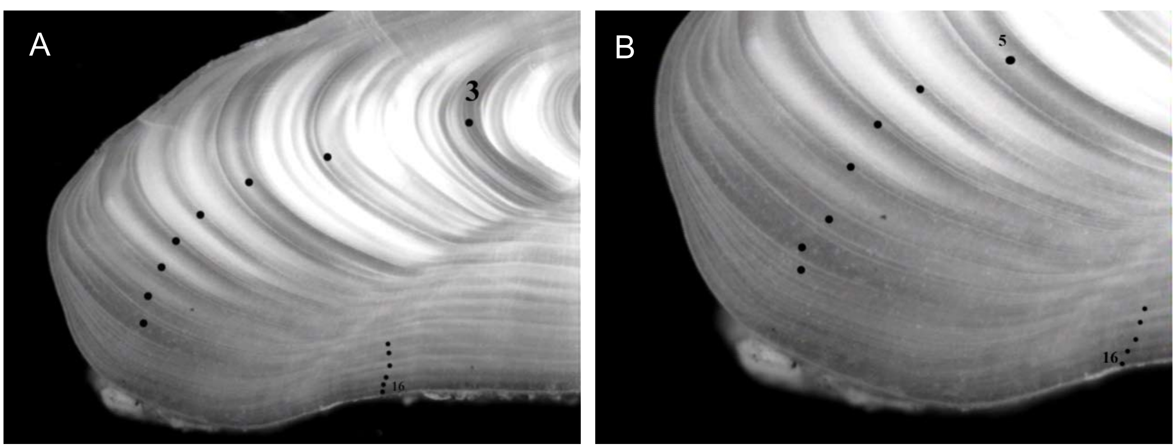


Fig. 35. An annotated photograph of a sectioned otolith from a 40 cm male captured in October/November 1999, showing an example of lateral growth at 20X magnification (**A**) and 40× magnification (**B**). Annuli are indicated by dots and numbers indicate the age.

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. 42.

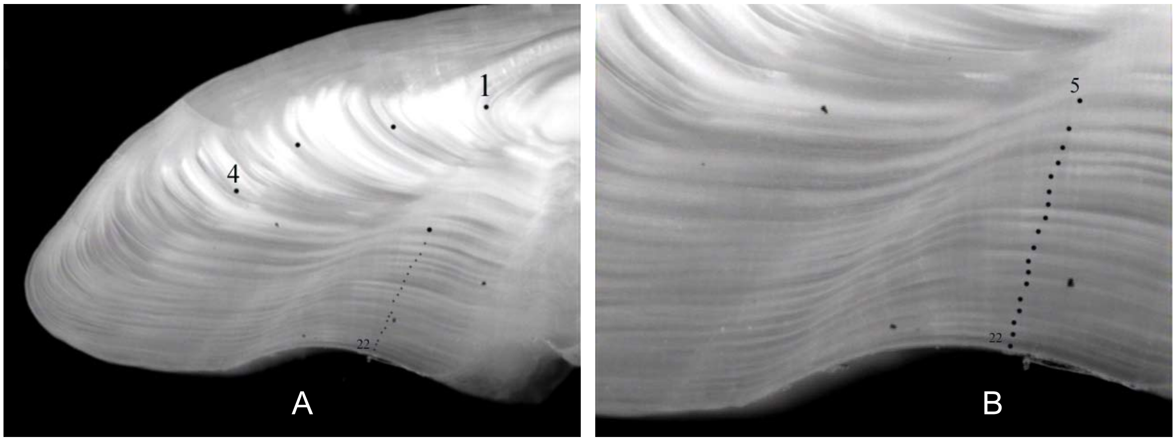


Fig. 36. An annotated photograph of a sectioned otolith from a 56 cm female (unknown capture date), showing an example of lateral growth at 20× magnification (**A**) and 40× magnification (**B**). Annuli are indicated by dots and numbers indicate the age.

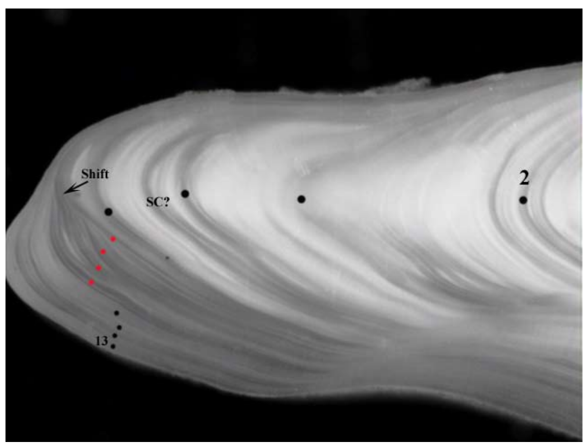


Fig. 37. An annotated photograph of a sectioned otolith from a 50 cm male captured in November 1988, showing an example of a "shift" pattern that includes several years bunched into one. Annuli are indicated by dots and numbers indicate the age.

There are some otolith sections that have an unusual shape (Fig. 43). Not only is the growth along Axis I longer, 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). It is possible that these otoliths come from yellowtail flounder that possibly mature later than average, and therefore do not show slowing in growth as early.

Edge Type

The edge type of the otolith, either opaque (summer) or translucent (winter) growth, and the amount of it, is important for determining the year in which the annulus has formed (age designa-

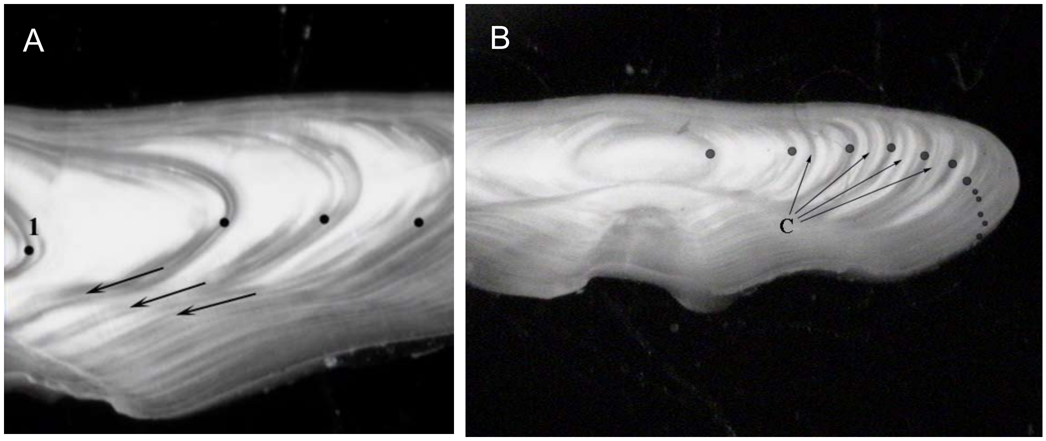


Fig. 38. Annotated photographs of sectioned otoliths, showing an apparent "doubling" or "splitting" of the annuli which occasionally occurs in samples. This may be a problem in older fish when it is more difficult to separate the annuli, because the annuli are more narrow. The "doubling" can be seen in the *sulcus* area of this otolith, from a 42 cm male flounder, captured in October/November 1999 (**A**). There appears to be a number of "split" annuli in the otolith from a 52 cm female captured in May 1983 (**B**). Knowledge of growth patterns can help a reader determine whether a ring is a check or an annulus.

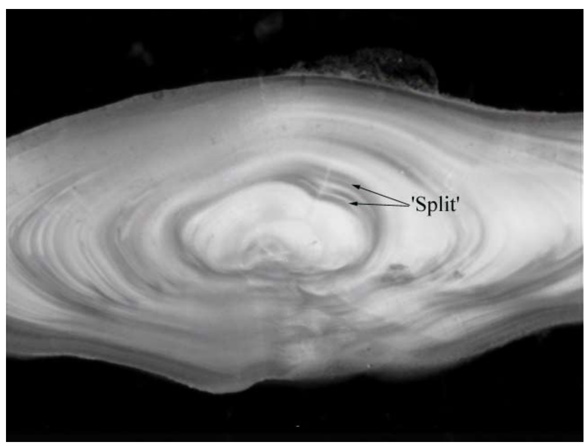


Fig. 39. An annotated photograph of an otolith section from a 27 cm female captured in June 1998 showing a "split" first annulus.

tion). Typically the annulus is finished forming in spring (March–April) in cold water flatfish, and opaque growth forms throughout the summer and autumn 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 (NO), Wide Opaque (WO), Narrow Translucent (NT) or Wide Translucent (WT) and should be compared to the amount of material that has been laid down in the previous year. In winter, the edge of the otolith typically displays a WT edge; in spring, a NO edge; summer, a WO edge and autumn, a NT edge (Fig. 44). The edge type will be used to assign an age to the otolith.

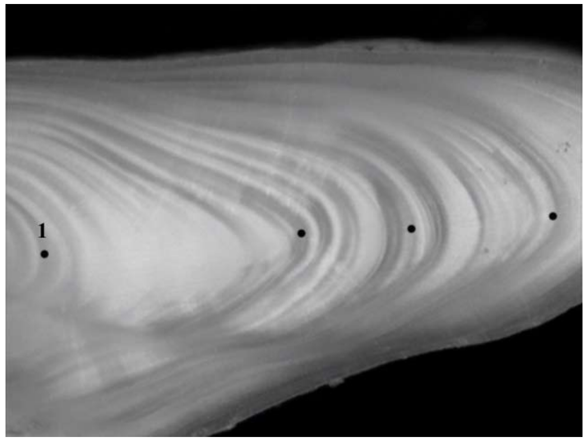


Fig. 40. An annotated photograph of a "checky" otolith. This otolith is from a 22 cm female captured in November 2000. The checks seen here would be grouped or lumped together to be considered one annulus.

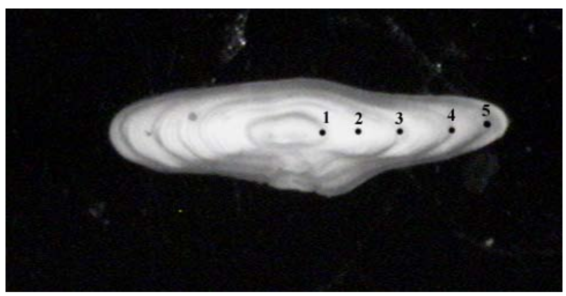


Fig. 41. An annotated photograph of a sectioned otolith from a 25 cm female captured in July 1999 and aged as 5-year. The second annulus is vague, but was confirmed by its better appearance on the ventral side of the otolith.

If the reader counts 4 annuli with opaque growth in otoliths collected in the spring, the age assigned will be 5 years. The same otolith in the autumn will be called 4 years. Typical edge growth is described in Fig. 44.

Trouble-shooting the Thin-Sectioning Method

There are some problems using the thin-sectioning method. Most of these problems occur because of an improper section (not cut directly through the nucleus). Some areas, when sectioned, appear "cloudy" or the blade leaves an imprint in the section. Annuli within that area are easily misinterpreted (Fig. 45) and this happens most frequently in the *sulcus* area. Using a polishing film to enhance annuli or remove any residue may help, but in most cases, when care is taken to section through the annulus, this will not happen.

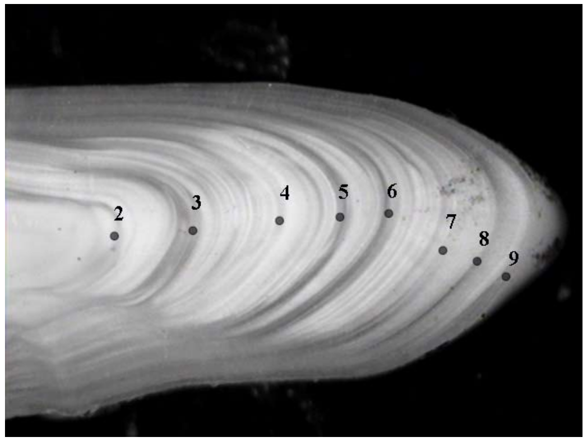


Fig. 42. An annotated photograph of a sectioned otolith from a 32 cm female captured in November 2000. The 4th annulus is vague but if it was not counted, the pattern of growth between 3 and 5 would be too wide, based on knowledge of growth patterns. When the weak 4th annulus is followed into the sulcus, it looks more prominent. Between annuli 5 and 6, although spacing is irregular between these years, they would both be counted as annuli. This is an example of the somewhat atypical situation in which the growth increment between two earlier years (5 and 6) is less than between older years (6 and 7).

Fig. 43. An annotated photograph of a sectioned otolith to show an example of an unusual shape. This otolith was taken from a 47 cm female captured in October/November 1999.

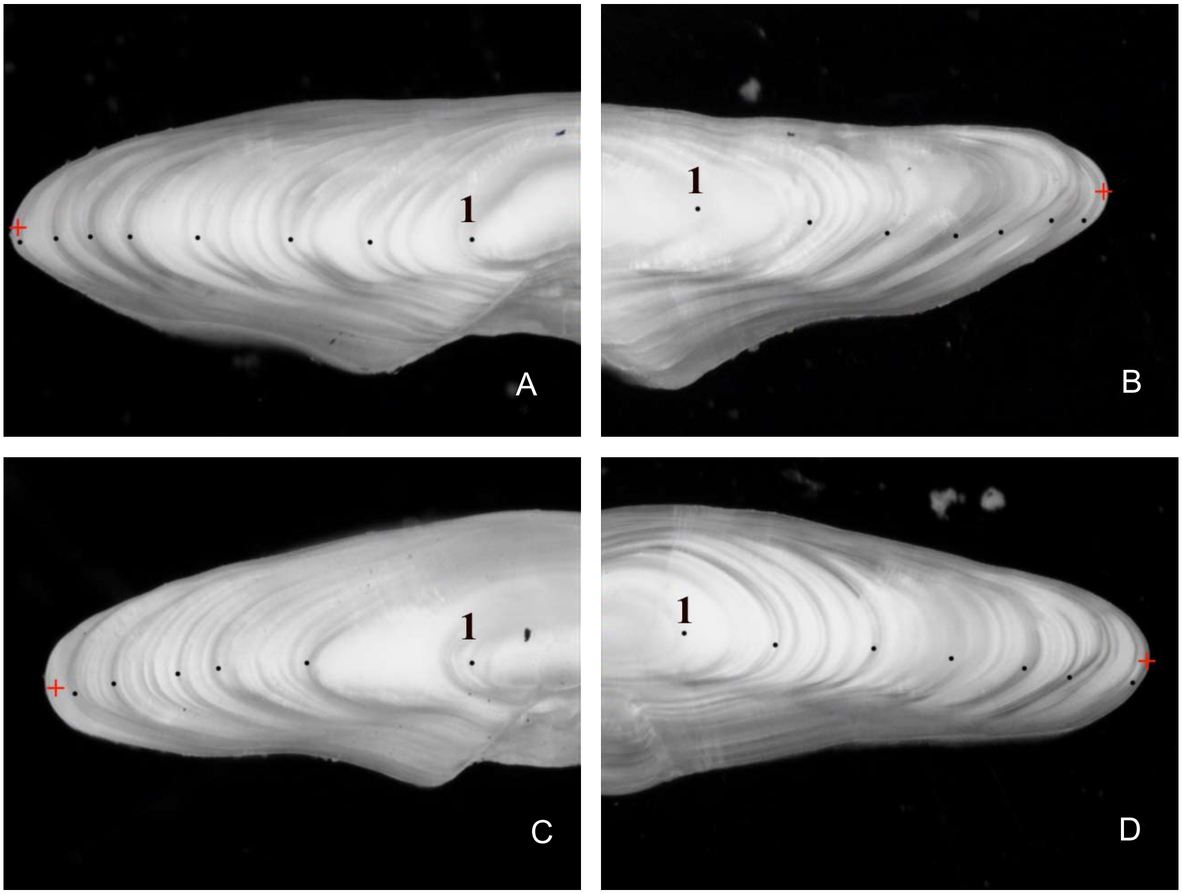


Fig. 44. Annotated photographs of sectioned otoliths showing edge growth for the four seasons. Although there is variation in each season, the otolith edge growth is generally indicated with crosses to indicate the edge type of each otolith. Panel A shows an otolith from a 25 cm female captured in February 1985 and forming winter growth. It is classified as having a wide translucent edge (WT). Panel B shows an otolith from a 25 cm male 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 (NO). Panel C shows an otolith from a 26 cm male with the edge forming the summer zone at the time of capture in August 1985. Its edge is classified as wide opaque (WO). Panel D shows an otolith from a 28 cm male captured in October 1985, which was in the process of starting to form winter growth. Its edge is classified as narrow translucent (NT).

If the otolith is not lined up directly through the nucleus, the section will miss the first annulus and it will either not be counted or it will be estimated. The series of sections 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.

Validated Images

Any new method of age determination should be validated for accuracy. There are many types of validation studies that can be carried out. Dwyer *et al.* (2003) carried out age validation studies on thin-sectioned otoliths for ageing yellowtail flounder, and this proved the accuracy of this method for ageing this stock of fish. This section shows some images that were validated in the study us-

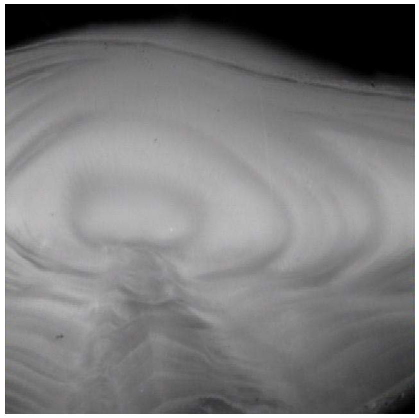


Fig. 45. A photograph showing a sectioned otolith that has been cut inaccurately, resulting in a "cloudy" area. Sometimes this is in an area that obscures the reading of the annuli. This otolith was taken from a 49 cm female captured in August 1988.

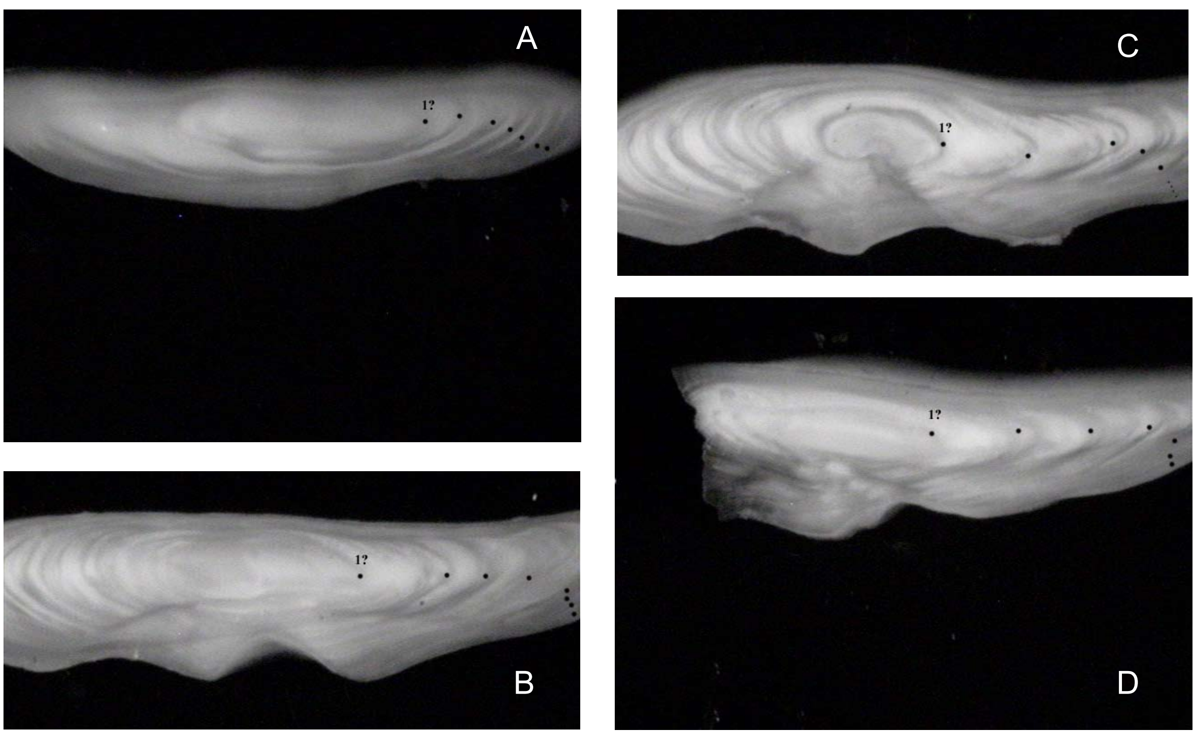


Fig. 46. Annotated photographs of a series of sections taken through the same otolith, demonstrating what happens when the otolith is not lined up properly for sectioning. In Panels **A** and **B**, the cut made by the saw has not cut through the nucleus, and in only one of the series (Panel **C**) did the nucleus get sectioned. If the otolith is not cut correctly, it is possible to miss the first annulus, and as a result, the otolith will have an inaccurate count. This otolith was taken from a 44 cm female yellowtail flounder and was captured August 1999. The inaccurate cutting 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 annular zone relationships. Thus, for example, in Panel **D**, a reader should be able to tell that one annulus cannot be seen and adjust accordingly.

ing bomb radiocarbon assays (Dwyer *et al.*, 2003) and can serve as a guide for age determination of sectioned otoliths. 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.

By following the growth patterns and determination of annuli from these photos (Fig. 47–49), it can help an age reader with their own ageing. In addition, the use of software such as Photoshop

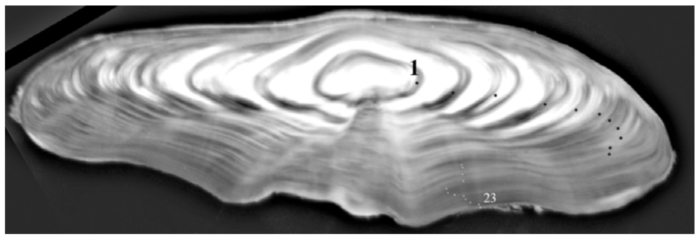


Fig. 47. A photograph of a sectioned otolith from a 51 cm female captured in April 1980, that was given an age of 23-years. Dots and numbers indicate the number of annuli and the age, respectively.

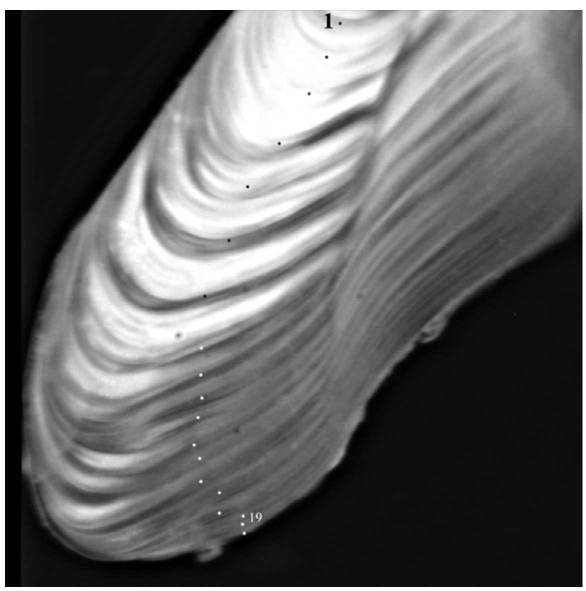


Fig. 48. An annotated photograph of a sectioned otolith from a 50 cm female captured in October 1972, that was given an age of 19 years. Dots and numbers indicate the number of annuli and the age, respectively.

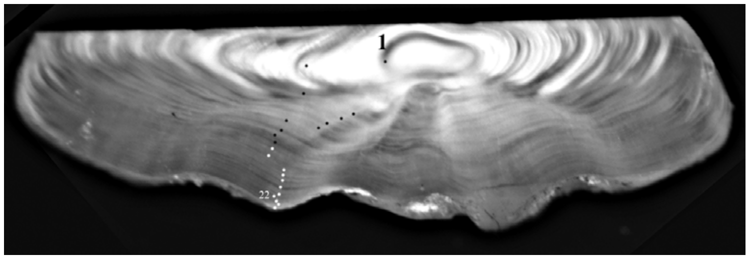


Fig. 49. A photograph of a sectioned otolith from a 54 cm female captured in October 1972, that was given an age of 22 years. Dots and numbers indicate the number of annuli and the age, respectively.

can help digitally enhance images on a computer screen which in turn may aid in the ageing of particularly difficult otoliths.

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. 50).

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. 51).

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

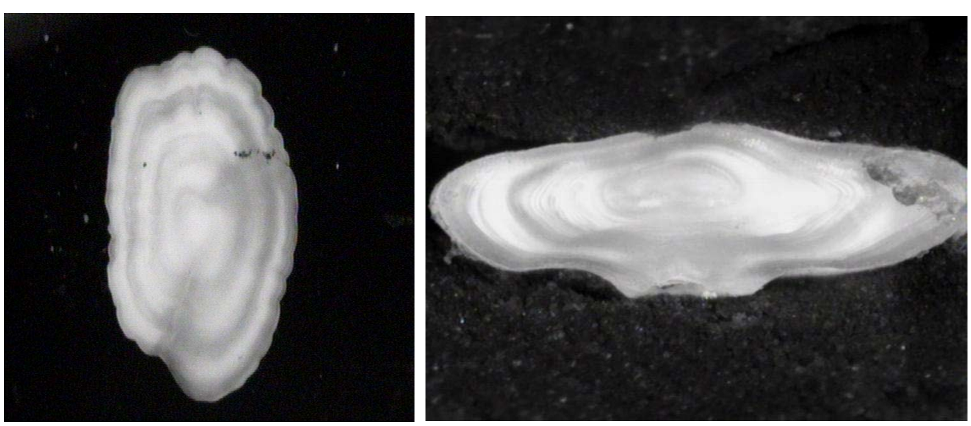


Fig. 50. Photographs of whole and sectioned otolith from inshore yellowtail flounder. Panel **A** shows a whole otolith from a 14 cm male showing the wide annuli and scalloped edge. The wide annuli can also be seen in the sectioned otoliths as well. Panel **B** shows a sectioned otolith from a 12.5 cm female. Both fish were captured in April 1999 in Witless Bay, Newfoundland.

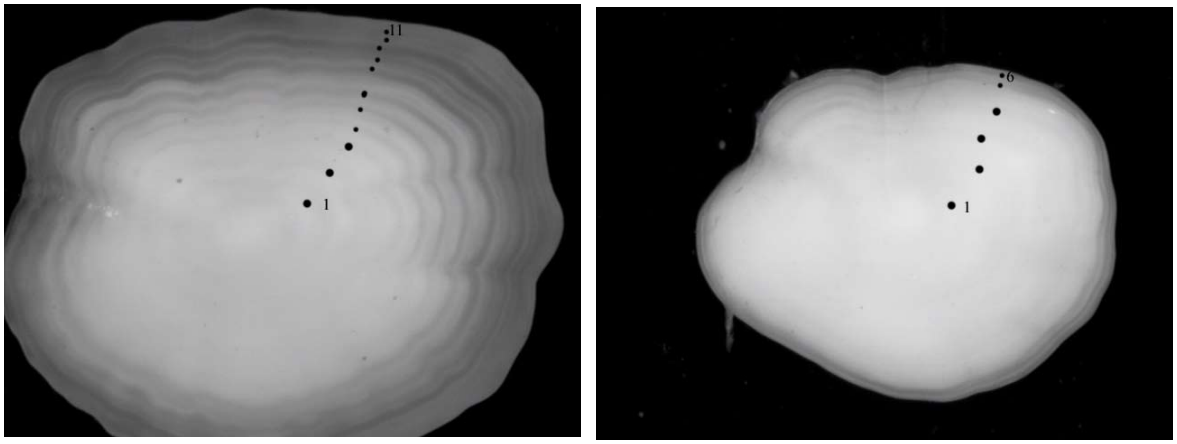


Fig. 51. Photographs of otoliths from 30 cm females taken under 7.5× magnification. The otolith was collected from an inshore yellowtail flounder in April 1999 (**A**), and in (**B**), the otolith was collected from an offshore (Grand Bank) yellowtail flounder captured in October/November 1998. The age determined from whole otolith from the inshore fish was 11 years, while the age from the offshore fish was 6 years. Dots and numbers indicate the number of annuli and the age, respectively.

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 of determining age for yellowtail flounder at present.

Knowledge of typical growth patterns of yellowtail flounder is important for ageing this species and aids the reader in assigning an age class for each fish. Mainly an age reader can improve technique of ageing by practice, and especially using a reference collection to increase precision.

Acknowledgements

Thanks to Drs Steve Walsh and Steve Campana for helping with yellowtail ageing work. 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 and Mick Veitch for locating some samples.

Glossary

(modified from Penttila and Dery (1988) and Walsh and Burnett (2002))

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

Annulus – any zone which forms once a year in a fish "hardpart", 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 composed either fully or partially of *vatarite*, a structural variant of aragonite, displaying what is generally assumed to be inadequate calcification. Age determinations are generally not possible due to lack of clarity of the 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 of an otolith representing the most recent growth.

Nucleus – central portion of an otolith.

Opaque zone – a zone that inhibits the passage of light in an otolith viewed under a microscope. With transmitted light, opaque zones appear dark and 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 viewed under the microscope.

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 in the otolith 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 – an illumination used from below an object used to pass through the object viewed under the microscope.

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 in a "hardpart". Synonymous with ring, band or mark.

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Information for Preparing Manuscripts for NAFO Scientific Publications

Introduction

The manuscript should be in English. The sequence of the material should be: title page, Abstract, text including Introduction, Materials and Methods, Results, Discussion and Acknowledgements and References. Number all pages, including the title page, consecutively with arabic numbers in the center of the top margin. There is usually no page limitation or page charge for accepted publications.

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This page should contain the title, followed by the name(s) and address(es) of the author(s) including professional affiliation, and any related footnotes. The title should be limited to what is documented in the manuscript and be as concise as possible. Where necessary the scientific names of species should be included.

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An informative abstract must be provided, which does not exceed one double-spaced page or about 250 words, the ultimate length being dependent on the size of the manuscript. The abstract should concisely indicate the content and emphasis of the paper. It should begin with the main conclusion from the study and be supported by statements of relevant findings. The scientific names of species where necessary should be included here. It is important that the abstract accurately reflect the contents of the paper because it is often separated from the main body of the paper by abstracting and indexing services.

Text

In general, the text should be organized into Introduction, Materials and Methods, Results, Discussion, Acknowledgments and References. Authors should be guided by the organization of papers that have been published in the NAFO Journal or Studies and by such authorities as the Council of Biological Editors Style Manual (CBE, 9650 Rockville Pike, Bethesda, MD 20814, USA).

The **Introduction** should be limited to the purpose and rationale of the study. The article should begin with a clear description of the subject (include where necessary the scientific names of species), stating the hypothesis and/or defining the problem(s) the research was designed to solve. Define the time of the study, along with literature review and other information limited to what is relevant to the problem.

The **Materials and Methods** should provide the framework for obtaining answers to the problems which concern the purpose of the study. Describe in sufficient detail the materials and methods used so as to enable other scientists to evaluate the work or replicate the work.

The **Results** should answer the questions evolving from the purpose of the study in a comprehensive manner in an orderly and coherent sequence, with illustrative tables and figures. Ensure only relevant information is presented to substantiate the findings. Avoid any confusion between facts and inferences and the restatement of table and figure captions in the text.

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The **Acknowledgements** should be limited to the names of individuals who provided significant scientific and technical support, including reviews, during the preparation of the manuscript, and the names of agencies which provided financial support.

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When preparing figures, consideration should be given to details such as shading and lettering with respect to the effects of reduction in size to a page width (e.g. lettering should not be overbearing or too small). If oversized figures are necessary, only good quality page-size photocopies should be submitted. If the paper contains photographs, ensure they have good contrast whether they are in colour or black and white.

Mathematical equations and formulae must be accurately stated, with clear definitions of the various letters and symbols. If logarithmic expressions are used, the type of function (e.g. log, ln, log_{10} or log_e) must be clearly indicated.

Manuscript Submission

NOTE: The following are the two major NAFO scientific publications, while the Scientific Council Research Documents (SCR Doc.) and Summary Document (SCS Doc.) are series that are submitted for meeting considerations.

The NAFO Secretariat now prefers to receive manuscript submissions, for any of the above publications in a computer electronic form.

The manuscript submissions may be done by e-mail (with a hard copy and diskette also forwarded by mail), or by mail (one hard copy and diskette). All texts, Tables and Figures should be formatted using Word or WordPerfect (Word is preferred), with each Table and Figure saved in a separate file (eps (preferably), tiff, pct, jpg, bmp or gif).

Please note: *In view of the considerable costs involved in colour printing, the NAFO Secretariat will be printing figures not requiring full colour in grayscale unless colour contributes to the content of the figure.*

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The Journal provides a forum for the primary publication of original research papers. While it is intended to be regional in scope, papers of general applicability and methodology, irrespective of region, may be considered. Both practical and theoretical papers are eligible. Space is also provided for notes, letters to the editor and notices.

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Manuscripts (one hard copy and one copy saved on a computer diskette) being submitted should be addressed to:

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Scientific Publications of the Northwest Atlantic Fisheries Organization

Journal of Northwest Atlantic Fishery Science

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This publication replaced *ICNAF Statistical Bulletin* which terminated with Vol. 28 (revised). The volume numbering continues the series as the *NAFO Statistical Bulletin*.

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This publication contains lists of all documents along with a subject and author index of the NAFO Scientific Council documents issued during 5-year periods.

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