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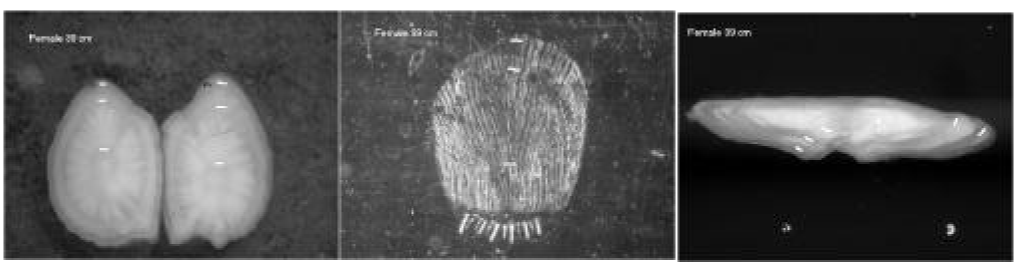
Report of the Canada-United States Yellowtail Flounder Age Reading Workshop
November 28-30, 2000, St. John's Newfoundland

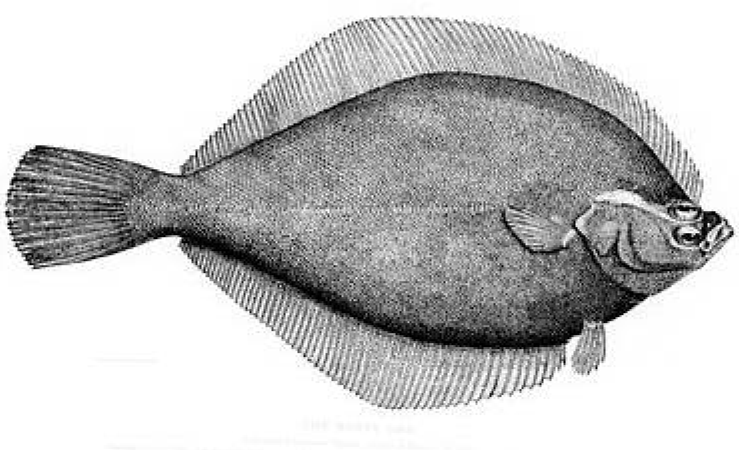
Editors

Stephen J. Walsh, Northwest Atlantic Fisheries Science Center, Dept. of Fisheries and Oceans, P.O. Box 5667, St. John's, Newfoundland Canada, A1C-5X1

and

Jay Burnett, Northeast Fisheries Science Center, National Marine Fisheries Service, 166 Water Street, Woods Hole, Massachusetts, 02543 United States





Abstract

This report records the presentations and discussions from the Canada-United States Yellowtail Flounder Age Reading Workshop held in St. John's, Newfoundland November 28-30 2000. Participants were from the Northwest Atlantic Fisheries Center, the St. Andrews Biological Station, the Bedford Institute of Oceanography, the Gulf Fisheries Center, The Northeast Fisheries Science Center, Newfoundland Inland Fish and Wildlife Division and the Massachusetts Division of Marine Fisheries. The workshop reviewed the current methods and structures used in aging yellowtail flounder in eastern Canada and the United States. Topics included biology, standardization of terminology, standardization of reading protocols, comparison of ages from both otolith and scales, new techniques to prepare samples, statistics, age validation, training, otolith/scale exchange programs and reference collections. As a result, the workshop made 15 recommendations to address various issues. It concluded that slow growing cold-water species are often underaged using the whole otolith method. Progress on implementation of these recommendations will be reviewed in 3 years.

1. Introduction

In recent years, the accuracy of determining the ages of older yellowtail flounder at the Northwest Atlantic Fisheries Center (NAFC), Newfoundland Region of the Department of Fisheries and Oceans (DFO) Canada, has been in doubt. In 1999, communications were established with other yellowtail researchers involved in aging yellowtail flounder at DFO institutes in eastern Canada and also in the Massachusetts area of the eastern United States. Age readers at Northeast Fisheries Science Center (NEFSC) typically used scales while NAFC typically used otoliths to age yellowtail flounder. Researchers at the St. Andrews Biological Station (SABS) who are involved in joint stock assessment of the trans-boundary (Canada/United States) Georges Bank stock had recently begun aging yellowtail flounder using otoliths. Researchers at the Moncton Gulf Fisheries Center (GFC), responsible for assessing the Southern Gulf of St. Lawrence Stock and the Dartmouth Bedford Institute of Oceanography (BIO), responsible for assessing Scotian Shelf stocks were interested in starting aging programs in the near future.

In 2000, funds were secured from NAFC to host a workshop with the specific aim of reviewing the current age reading protocols for yellowtail flounder at each institute (see Appendix I for workshop agenda)

1.1 *Terms of reference*

- Evaluate the various structures and methods used to age yellowtail flounder in various regions of eastern Canada and the United States;
- Develop standardized protocols for age estimation, training, testing and consistency of evaluation;
- Establish a mechanism for inter-lab exchange of age estimation material; and
- Record workshop proceedings and report to NAFO Scientific Council in June 2001

1.2 Participants (see Appendix VII)

Steve Walsh, Northwest Atlantic Fisheries Center (NAFC), St. John's (Convener)
Karen Dwyer, Northwest Atlantic Fisheries Center (NAFC), St. John's (Organizer)
Mick Veitch, Northwest Atlantic Fisheries Center (NAFC), St. John's
Rob Perry, Inland Fish and Wildlife Division (IFWD), St. John's
Jay Burnett, Northeast Fisheries Science Center (NEFSC), Woods Hole (Convener)
Vaughn Silva, Northeast Fisheries Science Center (NEFSC), Woods Hole
Jeremy King, Massachusetts Division of Marine Fisheries MDMF, Pocasset
Steve Campana, Bedford Institute of Oceanography (BIO), Dartmouth (Convener)
Mark Fowler, Bedford Institute of Oceanography (BIO), Dartmouth
Isabelle Forest, Gulf Fisheries Center (GFC), Moncton
Peter Perley, St. Andrews Biological Station (SABS), St. Andrews
Heath Stone, St. Andrews Biological Station (SABS), St. Andrews

2. Importance of age determination

Many analytical stock assessment models are age based and rely on accurate age determination of fish samples. Errors in age determination may cause inaccurate estimates of population numbers at age that can have serious repercussions for conservation of the resource and the viability of the fishing industry due to incorrect quotas. In addition to its fundamental importance to depict and forecast population trends, studies of biological parameters such as growth and maturity and estimates of natural and fishing mortalities also rely on the accurate assessment of age structure.

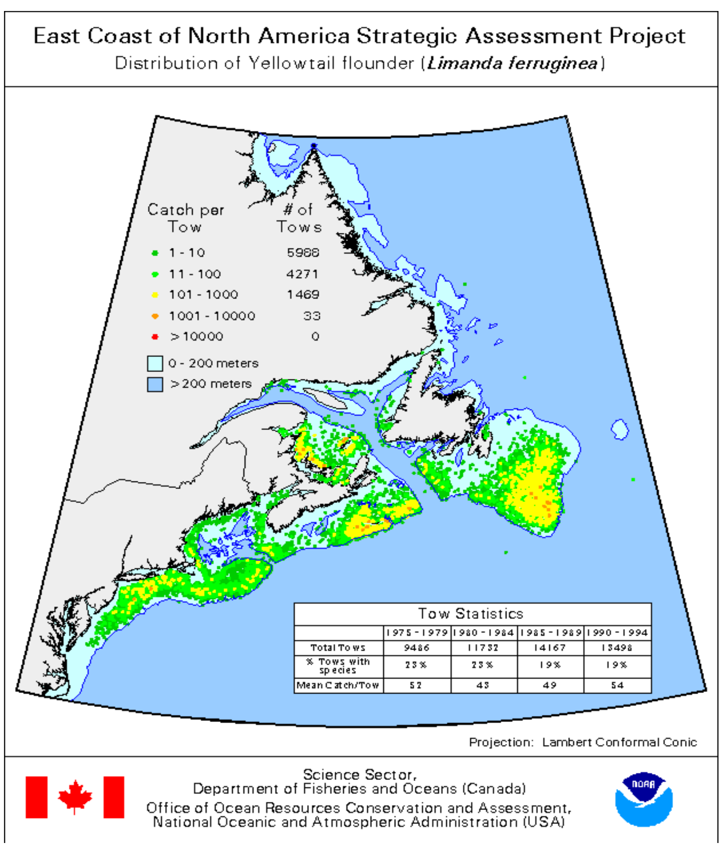
Age is normally obtained by reading scales and otoliths for annual increments. Whether these increments are actually annual requires age validation. In the Northwest Atlantic, yellowtail flounder are primarily aged by scale reading in the eastern United States and by otolith reading in eastern Canada.

3. General biology

On Day 1, by way of introduction, each institute presented an overview of age related research programs being currently carried out in their respective areas (see Appendix II for summaries)

3.1 *Distribution*

Yellowtail flounder (*Limanda ferruginea*) is a small mouthed right-eyed flounder distributed in the Northwest Atlantic from southern Labrador to Chesapeake Bay. The most northerly distribution of commercial quantities is found on the Grand Bank, Northwest Atlantic Fisheries Organization (NAFO) Div. 3LNO, off the coast of Newfoundland. There is a small, unregulated stock on the St. Pierre Bank off the south coast of Newfoundland. In the southern Gulf of St Lawrence, NAFO Div. 4T, yellowtail flounder are mainly concentrated around the Magdalen Islands and in the southern section of Div. 4T. On the Scotian Shelf, NAFO Div. 4VWX, it is most abundant on Banquereau and Sable Island Bank and the southern portion of Browns Bank. It is also found in smaller quantities in the Bay of Fundy. Off the northeast coast of the United States, four stocks have been identified based on larval distributions, historical tagging data, and geographical patterns of commercial landings and research vessel survey catch data (U.S. DOC 1998).



These stocks are located in the Mid-Atlantic region (NAFO Subarea 6), Southern New England (NAFO Div. 5Zw), Georges Bank (NAFO Div. 5Ze), and Cape Cod (U.S Statistical Reporting Areas 514 and 521). With the exception of the southern Gulf of St. Lawrence stock, which is primarily an industrial fishery for bait, yellowtail flounder is fished primarily for human consumption.

Yellowtail flounder are usually found aggregated on sand or sand/mud bottom at depths of 20-70 m (10-40 fathoms) and are capable of tolerating a wide range of temperatures, -1.2 to 15 ^{o} C, and salinities. In most stocks, seasonal movements are not extensive with the exception of the southern Gulf of St. Lawrence stock where yellowtail flounder migrate to shallower waters in the spring and deeper waters in the winter.

3.2 *Age, growth and maturity*

As with most flounders, yellowtail growth rates are sexually dimorphic, with males growing slower beginning with the onset of maturation, and to a smaller maximum size, than females. Across the entire range of the species, growth varies latitudinally, with faster growth but to a smaller maximum size occurring in southern regions off the US and slower growth to a larger maximum size characteristic of fish in northern regions, i.e. the Grand Bank stock. Within regions differences in growth are also evident. Yellowtail flounder can reach a maximum size of 58 cm and attain a weight of 1.2 kg. High fishing mortalities have reduced the relative abundance, average size and age in the population in several areas.

On the Grand Banks, historically, yellowtail have been aged up to age 14, although there is now growing evidence that suggests they may reach much older ages. Although a maximum age of 17 has been reported, few fish in U.S. waters exceed age 8 or 9. Yellowtail from the southern Gulf of St. Lawrence and the Scotian Shelf are presently not aged

On the Grand Bank, males mature at an median age (A_{50}) of 4 years (L_{50} = 25cm) and females mature at an median age of 6 years (L_{50} = 34 cm) (Walsh and Morgan 1998). In the southern range, the southern New England stock of male yellowtail flounder mature at an median age of 1.8 yr. (L_{50} = 19.6 cm) and females mature at an median age of 1.6 yr. (L_{50} = 25.5 cm). Georges Bank males mature at median age of 1.3 yr. (L_{50} = 21.4 cm) and females mature at 1.8 yr. (L_{50} = 25.8 cm). Cape Cod males mature at an median age of 2.6 yr. (L_{50} = 26.8 cm) and females also mature 2.6 yr. (L_{50} = 29.7 cm) (O'Brien 1993). Differences in median length at maturity between stocks reflect the differences in growth rates. Information on maturity is not available for the Scotian Shelf and southern Gulf of St. Lawrence stocks.

3.3 *Current age estimation methodologies at various institutes*

NAFC: Age samples from the Grand Bank stock date back to 1949 in the historic collection. The otoliths are stored dry in coin envelopes. Most otoliths are read whole, however troublesome and older fish otoliths are ground with a flat abrasive stone. Both sex and length information are available. There is no distinction made on which otolith to read (i.e. left vs. right). Although the edge is read and coded it is not used. Birthday is January 1. Current sampling protocols during surveys are length stratified with 5 fish per sex per 2 cm group collected on the northern Grand Bank and 3 fish per sex per 2 cm group on the southern Grand Bank. In 2000, scales were collected during surveys for age reading comparison with otoliths from the same fish. In addition, both baking and sectioning otolith techniques were tested. Reference collection is currently under development.

GFC: Age samples from the southern Gulf of St. Lawrence date back to 1971 in the historic collection. The otoliths are stored dry in coin envelopes. However, age reading has not been a priority. Some age estimation was carried out between 1972-1987 and fish ages ranged from 2-14 years, however, the maximum age was generally around age 10 . Yellowtail flounder in the Gulf are small, maximum size is ~ 38 cm, and are mainly used for bait in other fisheries. In 1995, a biological update was started and is continuing, but age reading has not begun. It is intended that age estimation protocols established for American plaice be applied to yellowtail flounder. Whole otoliths are read. A reference collection is used by the primary age reader to calibrate their reading before beginning a survey collection. Length and sex are not available for the reader, however, month caught is. The edge is defined and the size calculated.

BIO: yellowtail flounder otoliths were collected during research surveys since 1970. Only otoliths collected from 1970-1983 have ever been read. Otoliths are stored in a glycerine/thymol solution. No maturity data has been collected since 1985. This species is assessed together with plaice and witch flounder as a flatfis h species complex.

SABS: Otoliths from Georges Bank have been collected since 1977 from port commercial samples and since 1987 from research vessel surveys. Commercial samples are stored dry in coin envelopes, while research survey otoliths up to 2000 have been stored in vials containing 70% glycerine and water solution to which 0.9 gm of thymol has been added to limit bacteria activity. This activity will now be discontinued since long-term storage in glycerine/thymol may cause over-clearing. During surveys a length stratified sampling routine is used and 1 set of otoliths is collected per 1 cm group per sex. In 2000 scales were collected during port sampling of landed catches for age reading comparison with otoliths from the same fish. No age estimation has been done prior to 2000. Currently, whole otoliths, sectioned otoliths and scales are being examined to determine which structure is the best for age determination.

NEFSC: Age and growth studies of yellowtail flounder in U.S. waters date back to the 1950s. Scott (1954) reported that, compared to those from Scotian Shelf fish, otoliths from Cape Cod fish were less desirable than scales as an age estimation structure. Royce et al. (1959) validated the use of scales for age determination using cohort analysis and marginal increment analysis; Lux and Nichy (1969) further verified scale-based age estimation with tag-recapture data and length frequency modal analysis. The Age and Growth Unit of the Northeast Fisheries Science Centre (NEFSC) has aged yellowtail using scales since 1956, and production age estimation of research vessel survey samples and commercial landings samples began in 1970. Details of age sample processing and age estimation methodology are given by Penttila (1988). During surveys an unsexed sample is taken at each station consisting of 1 fish per 1 cm grouping. The sampling scheme for commercial samples is 15 males and 15 females per 100 lengths. Both scales and otoliths (recent years) are stored dry. Two age readers are used: primary age reader completes 2 readings of the collection while the secondary reader reads 10-50% of the collection. Primary and secondary readers conduct percent agreement and age bias exercise. Reference collection is under development.

MDMF: Age estimation of Cape Cod yellowtail flounder is carried out using scales following those procedures used at NEFSC. Including surveys completed in 2000, 4,836 age samples have been collected on spring surveys and 2,599 samples collected in the autumn. A subset of age samples from 20 spring cruises indicates that fewer than 5% of samples collected represent fish older than age 4. VPA results 1985-1999 indicate that the commercial catch is mostly (93%) composed of ages 2 – 4 (Cadrin, 2000)

3.4 *General discussion*

A summary table outlining the regional differences in scales and otolith used is listed below.

Table 1 Various preparation techniques currently use by the different institutes.

Lab	Both otoliths removed	Scales taken	Stored dry	Stored in glycerin: thymol solution
NAFC	x		x	
NEFSC	x	x	x	
SABS	x	x	x	x[1]
GFC	x		x	x[2]
MDMF		x	x	

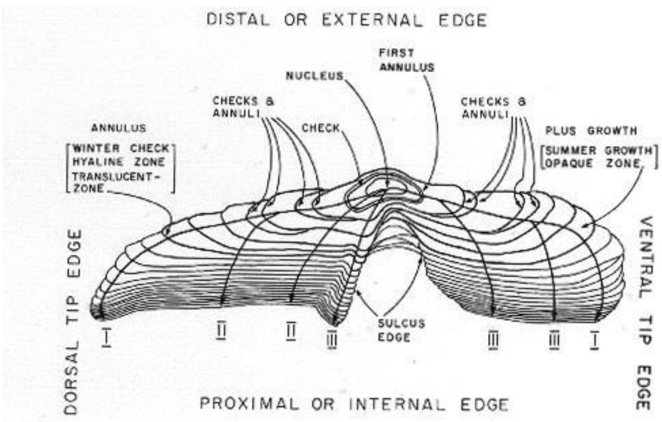
[1]stopped in 2000
[2]stopped in 1993

The ***workshop noted*** that shrinking budgets and resources have deteriorated the quality control in age reading in Canadian institutes. The existence of age estimation material for each institute is listed in Appendix III. Most of the Canadian otoliths have not been aged.

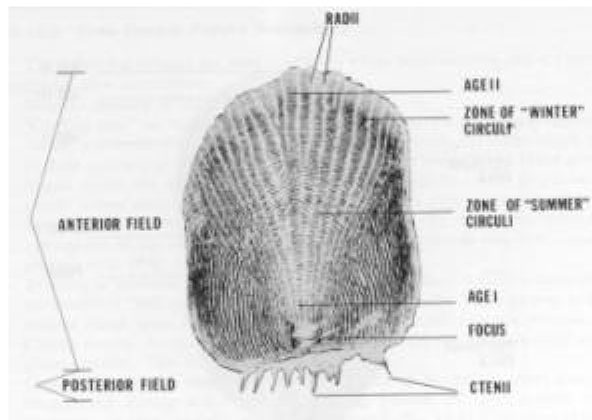
4. Standardization of age reading terminology

The workshop reviewed the glossary for otoliths (Secor et al. 1995) and scales (Penttila 1988) and recommended that the following definitions be used when making reference to yellowtail flounder otoliths and scales. Specific references to scales are in italics.

The following figure illustrates some of the morphology of the common otolith and scale



Otolith (MacKellan, 1997)



Scale (Jearld, 1983)

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

2. **Age estimation, age determination** – these terms are preferred when discussing the process of assigning ages to fish. The term aging (ageing) should not be used as it refers to time-related processes and the alteration of an organism's composition, structure and function over time. The term age estimation is preferred.

3. **Age group** – the cohort of fish that have a given age (e.g. the 5-year-old age-group). The term is not synonymous with year-class or day-class.

4. **Annulus** - one of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent or opaque zone that can be seen along the entire structure or as a ridge or a groove in or on the structure. In some cases, an annulus may not be continuous or obviously concentric. The optical appearance of these marks depends on the otolith structure and the species and should be defined in terms of specific characteristics on the structure. This term has traditionally been used to designate year marks even though the term is derived from the Latin "anus" meaning ring, not from "annus", which means year. The variations in microstructure that make an annulus a distinctive region of an otolith are not well understood . *For yellowtail flounder scales, the annulus is defined as a zone of close winter circuli marking the end of a year of growth, i.e. the winter growth zone.*

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

6. **Check** - a discontinuity (e.g. a stress-induced mark) in a zone, or in a pattern of opaque and translucent zones, or microincrements. Microstructural checks (eg. hatching checks, settling checks, spawning checks) often appear as high-contrast microincrements with a deeply etched D-zone or an abrupt change in the microstructural growth pattern. If the term is used, it requires precise definition. *For yellowtail flounder scales, checks are*

defined as a zone of slow "winter" type growth which is not a true annulus. Such rings are distinguished by the width of the zone relative to annuli, location relative to annuli, and incomplete formation or poor definition.

7. **Circulus**-*in scales, a concentric ridge formed on a scale by the periodic addition of material to the edge of the basal plate.*

8. **Cohort** - a group of fish of a similar age that were spawned during the same time interval. Used with both age-group, year-class, and day-class.

9. **Corroboration**-a measure of the consistency or repeatability of an age determination method. For example, if two different readers agree on the number of zones present in a hard part, or if two different age estimation structures are interpreted as having the same number of zones, corroboration (but not validation) has been accomplished. The term verification has been used in a similar sense; however, the term corroboration is preferred as verification implies that the age estimates were confirmed as true.

10. **Ctenoid scale**-*type of scale having ctenii, or spine-like projections, on its posterior edge. Yellowtail flounder scales are ctenoid.*

11. **Cutting-over**-*in scales, disruption of the circulus pattern from erosion of the edge, resulting in circuli that appear to intersect or "cut over" previously-formed circuli. If scale edge erosion is an annual event, the "cutting-over" marks may be used to detect annuli. For yellowtail flounder, cutting-over marks in the lateral fields can help verify annulus identification.*

12. **Focus**-*in scales, the center or origin.*

13. **Hyaline zone** – a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone. Alternatively, it is known as the winter growth zone.

14. **Increment** – a reference to the region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (eg. subdaily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry and period of formation can vary widely. A daily increment consists of a D-zone and an L-zone, whereas an annual increment comprises an opaque zone and a translucent zone. Both daily and annual increments can be complex structures, comprising multiple D-zones and L-zones or opaque and translucent zones, respectively.

15. **Marginal increment (edge)** – the region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment. Precision is required when determining edge type, as qualitative errors may occur.

16. **Nucleus, kernel** – collective term originally used to indicate the primordia and core of the otolith. These collective terms are considered ambiguous and should not be used. The preferred terms are primordium and core (see definitions).

17. **Opaque zone** – a zone that restricts the passage of light when compared with a translucent zone. The term is relative because a zone is determined to be opaque on the basis of appearance of adjacent zones in the otolith (see translucent zone). In untreated otoliths under transmitted light, the opaque zone appears dark and the translucent zone appears bright. Under reflected light, the opaque zone appears bright and the translucent zone appears dark. An absolute value for the optical density of such a zone is not implied. It is also known as the summer zone. See translucent zone**.**

18. **Precision –** the closeness of repeated measures of the same quantity. For a measurement technique that is free of bias, precision implies accuracy.

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

20. **Regenerated scale**-*scale which replaces one previously lost. These scales cannot be used for age determination because the central area has no circuli or annular growth features.*

21. **Sagitta** – one of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes. It lies within the sacculus ("little sack") of the pars inferior. It is usually compressed laterally and is elliptical in shape; however, the shape of the sagitta varies considerably among species. In non-ostariophysan fishes, the sagitta is much larger than the astericus and lappilus. The sagitta is the otolith used most frequently in otolith studies.

22. **Sulcus acusticus** (commonly shortened to sulcus) – a groove along the medial surface of the sagitta. The thickened portion of the otolithic membrane lies within the sulcus acusticus. The sulcus acusticus is frequently referred to in otolith studies because of the clarity of increments near the sulcus in transverse sections of sagittae.

23. **Transition zone** – a region of change in otolith structure between two similar or dissimilar regions. In some cases, a transition zone is recognized due to its lack of structure or increments, or it may be recognized as a region of abrupt change in the form (eg. width or contrast) of the increments. Transition zones are often formed in otoliths during metamorphosis from larval to juvenile stages or during significant habitat changes such as the movement from a pelagic to a demersal habitat or a marine to freshwater habitat. If the term is used it requires precise definition.

24. **Translucent zone** – a zone that allows the passage of greater quantities of light than an opaque zone. The term is a relative one because a zone is determined to be translucent on the basis of the appearance of adjacent zones in the otolith (see opaque zone). An absolute value for the optical density of such a zone is not implied. In untreated otoliths under transmitted light, the translucent zone appears bright and the opaque zone appears dark. Under reflected light, the translucent zone appears dark and the opaque zone appears bright. The term hyaline has been used, but translucent is the preferred term.

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

26. **Validation** – the process of estimating the accuracy of an age estimation method. The concept of validation is one of degree and should not be considered in absolute terms. If the method involves counting zones, then part of the validation process involves confirming the temporal meaning of the zones being counted. Validation of an age estimation procedure indicates that the method is sound and based on fact.

27. **Verification** – the process of establishing that something is true. Individual age estimates can be verified if a validated age estimation method has been employed. Verification implies the testing of something, such as a hypothesis, that can be determined in absolute terms to be either true or false.

28. **Year-class** – the cohort of fish that were spawned or hatched in a given year (eg. the 1990 year-class). Whether this term is used to refer to the date of spawning or hatching must be specified as some high-latitude fish species have long developmental times prior to hatching.

29. **Zone** – region of similar structure or optical density. Synonymous with ring, band, and mark. The term zone is preferred.

5. Standardization of age reading protocols

The workshop began a lengthy discussion on various sampling and storing procedures, preparation techniques and reading protocols. The workshop agreed that the following standard protocols be used when making reference to yellowtail flounder scale and otoliths and interpretation of their ages:

- *Prior information on fish length* – after some debate it was felt that having this information was a matter of choice.

- *Which otolith, left or right*- it was recommended that the left otolith be the standard otolith since this is widely used by most participants. The right otolith could be used as a verification of the age determination. *Which side of otolith, dorsal or ventral* – after much debate it was felt that most people used the ventral side.

- *Position angle of light and type of light* (reflective versus transmitted) – most participants used reflective light and recommended that be the accepted standard. *Magnification* – lower magnification in the range of 8-16X was required for whole otoliths and a higher magnification in the range of 25-40X was generally used for sectioned otoliths.

- *Axes to count along* – the common axis was along the longitudinal of the widest section of the otolith. Many participants use both sides because of regional differences in otolith structure.

- *Definition of nucleus* – it was critical that sectioning cut transversely though the otolith to see the nucleus. After sectioning both remaining halves should be retained for further examination if possible.

- *Determination of first annulus criteria* – the correct interpretation is a critical requirement for precise age reading. This is not a problem in scales. The extent of the second years growth was seen to differ in sizes for all stocks considered being wider in the south and narrower in the north.

- *Edge (marginal) growth* –new growth at the edge of the otolith or scale should be related to the time of the year the fish was sampled and adjusted according to the accepted birthday convention of January 1. Edge growth may be difficult to interpret in older fish. An accepted rule of thumb regarding the qualitative definition of wide and narrow growth zones: wide growth should be greater than 50% of the previous comparable zone and narrow less than 50 %.

- *Storage* - it was recommended that otoliths be stored dry. It was agreed that otoliths stored in glycerine could lead to a deterioration in clarity of the structure. When collecting scales at sea it is recommended to scrape off skin mucus before collecting scales to ensure better quality and these should be stored dry. The use of alcohol, detergent, mineral oil or clove oil to enhance clarity during reading was felt not to have any long term effect when the otolith was stored back into the envelope. It was recommended that otoliths could be soaked in glycerine/thymol for short periods till age determinations were complete, but after this they should be transferred to coin envelopes for long term dry storage.

6. Age reading comparisons between scales and otoliths

On Day 2, presentations were made by the three institutes that carried out age reading comparisons of scales and otoliths using various techniques before arriving at the workshop (see Appendix IV for details).

6.1 *NEFSC (Jay Burnett):* Yellowtail flounder in U.S waters have historically been aged using scales. As preparation for the Canada-US Yellowtail Flounder Age Estimation Workshop, the Age and Growth Unit of the Northeast Fisheries Science Center conducted a comparison study using scales, whole otoliths, and otolith thin-sections from individual fish collected during the spring and autumn 2000 bottom trawl surveys in the southern New England-Georges Bank region (NAFO Subarea 5). Scale and otolith preparations followed procedures described by Penttila et al. (1988); scales were aged using the methodology reported by Penttila (1988), and otoliths were aged using methods described for American plaice by Dery (1988).

Percent agreement of age determinations from each structure comparison was as follows:

Comparison	Spring Samples (n=41)	Autumn Samples (n=122)
Scales vs. whole otoliths	92%	64%
Scales vs. thin-sections	87%	81%
Whole otoliths vs. thin-sections	97%	74%

Although samples sizes were small, and U.S. age readers had no prior experience with the use of otoliths for yellowtail age determination, some general conclusions were made:

1) otoliths from Georges Bank yellowtail flounder were difficult to age, more so than those of other flatfish in that region (American plaice, witch and winter flounder). Rapid growth was noted between ages 1-2, accompanied by the presence of several strong checks in the corresponding opaque zone.
2) due to their thickness and diffuseness, whole otoliths were less desirable than thin-sections. The thickness often obscured the second annulus, which was clearly visible in thin-sections. For most instances in which age determinations differed between scales and thin-sections, the scale age was one year higher.
3) while thin-sections appear to be a superior structure for the age estimation of older fish in general, the use of scales for age determinations of yellowtail flounder in U.S. waters does not appear to be problematic given the current age composition of the stocks. However, as stock rebuilding continues on Georges Bank, the concomitant increase of older fish may necessitate the use of otolith thin-sections in the future.

<u>Discussion notes</u>: There is confusion in age type for scales and otoliths. Scales develop winter edge before otoliths thus there is an apparent mis-match in timing. SABS suggested that soaking whole otoliths for 4-5 days in glycerine/thymol solution would improve the clarity. This is unnecessary for sectioned otoliths. There was some debate as to whether it is safe to store whole otoliths in glycerine for long term storage. Note that Scotian Shelf otoliths examined at this workshop were stored in glycerine for over 20 years. Despite this, it was recommended that otoliths could be soaked in glycerine for short periods until age determinations were complete, but after this, they should be transferred to coin envelopes for long-term storage. The ***workshop recommended*** that whole otoliths should be soaked in a glycerine/thymol solution prior to age reading.

6.2 *SABS (Heath Stone):* Age readers from the St. Andrews Biological Station conducted a comparison study of age determinations using whole otoliths, scales and otolith thin-sections for Georges Bank yellowtail based on 1998 spring survey and 2000 port sample collections. Although sample sizes were small, whole otoliths tended to be underaged relative to scales and otolith thin-sections after age three. Percent agreement between scales and otolith thin-sections was 67% and indicated less bias than other comparisons (i.e. whole otoliths vs thin-sections and whole otoliths vs scales). It was concluded that, given the high efficiency at SABS for rapid preparation of thin-sections, thin-sections should be used as the main structure for age determinations rather than reading ages from whole otoliths up to age 3. Thin sections would also be used to age older fish. The ***workshop recommended*** that age readers from the NEFSC would examine samples of otolith thin-sections from the Canadian fishery on Georges Bank for comparisons with ages determined by Canadian age readers.

<u>Discussion notes</u>: There is a need to establish whether whole otoliths can be used up to a certain age and then after that age use thin sectioning or go with all thin sections. It was noted that you can miss the nucleus in some otoliths when attempting to section many otoliths together.

6.3 *NAFC (Karen Dwyer):* Results from several age reading comparisons for Grand Bank yellowtail flounder sampled from groundfish surveys (i.e. between readers, left/right otoliths, structure type and preparation method) were investigated. It was noted that after age seven there was a departure in ages determined from whole otoliths and otolith thin sections, with whole otoliths having lower ages than thin sections. The right otoliths tended to yield lower ages than left otoliths since they have a tendency to become more compressed during growth. Baking the thin sections did not enhance the image or the ability to determine the ages. When scales were used for age determinations, it was difficult to interpret “edges” in older fish and many scales tended to be regenerated. While whole otoliths underestimated ages determined from scales, the latter underestimated ages determined from thin sections. In the comparison of ages determined from scales and thin sections from the otoliths it was found that beyond age 7 scales were underageing the fish when compared to thin sections from the otoliths. It is concluded that whole otoliths could be used for age determinations up to age 7, but after that, thin sections provided the most accurate ages (especially for older fish). In some cases there was difficulty in determining the location of the first annulus. This might be resolved by obtaining samples of young fish around their first birthday. Scales from Grand Bank yellowtail were examined by NEFSC age readers and were found to be fairly easy to read up to age 7.

Discussion notes: The difficulty with the first annulus needs to be resolved to decrease bias in the aging of younger fish. The ***workshop concluded*** that exchanges of scales with the NEFSC lab should continue.

6.4 *IFWD/NAFC (Rob Perry)*: This presentation described a method used for preparation of brook trout otolith thin sections and applied to yellowtail flounder otoliths. This method involved several steps which included sectioning otoliths embedded in resin, polishing, etching with acid solution and then making replicates using an acetate peel. Although time consuming, the method produced very clear images of otolith thin sections. This method would be useful for producing good quality images, particularly for older fish, but would be too time consuming for production aging.

Discussion notes: This is a very promising technique and more comparisons between this method and the traditional thin sections is required especially in light of the fact that the acetate peel ages are higher.

6.5 General Discussion

The workshop noted that there are regional differences in otolith shapes and scales. In general scales are acceptable for younger ages in the southern range, however, on the Grand Bank they have a more limited use because it is difficult to interpret “edges” in older fish and many scales tended to be regenerated. Whole otoliths are also acceptable for younger fish, however, the thin sectioning method is more promising for older fish in all areas. Inter-institute exchange of otoliths and scales are encouraged for comparison of results within stocks and between stocks

7. Accuracy, precision and quality control in age determination

As an introduction, Steve Campana (BIO) presented excerpts from a review paper that he is writing on the subject of accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. A synopsis is presented below (sections 7.1 to 7.4).

7.1 *Age validation* refers to the accuracy of the aging method, not the accuracy of the age of the fish. Consistency is not the same as accuracy. There are many examples of precisely wrong ages such as North Atlantic redfish, walleye pollock, mako sharks and Scotia-Fundy haddock. The validation method may not validate the absolute age but usually validates the frequency (periodicity) of formation of the annual growth increments. The validation method does not account for interpretation error and it can still produce wrong ages. This is why we need a reference collection.

There are three main age validation priorities: 1) validate absolute age, 2) determine increment periodicity across the entire age range, and 3) determine the age at first increment formation.

Several examples of age validation methods were presented and ranked from most desirable to least desirable:
Release of known age fish into the wild which can validate absolute ages.
Bomb radiocarbon dating of fish that were hatched prior to 1965.
Mark-recapture of chemically tagged fish such as those marked with oxytetracycline.
Radiochemical dating validates absolute ages and can be applied to long lived fish.
Aging of discrete length modes is probably best suited for younger fish.
Use of natural markers can validate absolute ages.
Marginal Increment Analysis validates growth increments but is really only suitable for fast growing fish. Older fish are always a problem.

Rearing in captivity is not ideal for validating annulus formation but good for daily increments estimation for fish of known age. Laboratory environments rarely replicate natural environments.

Since 1983, most age validation studies cited for otoliths have been based on marginal increment analysis. It is probably the most used and most likely to be abused of the age validation methods because of the difficulty of discerning the increment in older, slow growing fish. It also requires samples throughout the year to determine the seasonal timing of the marginal increment with age.

7.2 *Methods of age corroboration* (verification) support the age validation method and include the following:

- Comparison of growth rates from different methods (i.e. tag recapture analysis, following the progression of strong year classes, the use of length frequency analysis);
- Ralston method (use of daily increment widths to determine yearly age); and
- Elemental isotope cycles.

However, the following examples represent neither age validation or corroboration:

- Comparisons of different structures from the same fish, i.e. scales and otoliths;
- Otolith exchanges;
- Comparison among age readers; and
- Back calculation.

7.3 *Measures of precision:* the best include CV's, average percent error, and index of dispersion. Percent agreement was considered to be one of the worst because it varies so widely among species and among ages within a species.

7.4 *Steps to a successful aging program*

I. Choose appropriate structure(s) for age estimation;
II. Develop the aging method best suited to the age structure;
III. Carry out age validation studies;
IV. Prepare a reference collection and use it – an ideal reference collection derives from the age validation studies in Step III; and
V. Conduct a quality control program on your age reading.

7.5 *Quality Control program* ensures that age interpretations do not 'drift' through time; since the reference collection is of 'known' ages then age interpretations remain accurate, and not just precise. The QC program ensures that the age interpretations of different age readers are comparable and it also eliminates the need for secondary age readers.

<u>The components of a Quality Control program</u>

1. *Reference collection* – ideally consists of known-age (i.e. validated) otoliths or alternatively consists of otoliths aged by international recognized experts.
2. *Monitoring* – occasionally age a subsample from the reference collection, plus a subsample of recently aged otoliths. The reference collection has to be updated by adding otoliths to eliminate 'reader learning'.
3. *Age Bias graph and CV's* – tools used to evaluate results of monitoring. When there is a consistent deviation or trend there may be a problem.

The *Reference Collection* is useful for:

- ✓ Monitoring age estimation consistency over time – if the reference ages are accurate, then the monitoring will also assess accuracy;
- ✓ Training new age readers; and
- ✓ Testing for consistency among age readers.

7.6 *Presentation of current practices used in accuracy, precision, age validation and quality control programs at various institutes.*

Various institutes with established age reading programs presented results of their current practices (see Appendix V). These are summarized here.

7.6.1 *NAFC (Karen Dwyer):* Quality control measures for Grand Bank yellowtail flounder aging were summarized.

The current "Indirect Methods" involve 1) inspection of first annulus (measure thickness and weight of otoliths from young of the year fish), 2) length frequency analysis to track modal progressions to confirm ages 1 and 2, 3) marginal increment analysis of fish from seasonal surveys, 4) determination of first annulus and 5) comparisons of wild and cultured fish to determine annual periodicity of increments. In terms of "Direct Methods", to date, there has only been some analysis of tagging recapture data. Tagging has revealed, however, that time at liberty corroborates ages obtained by using thin sectioned otoliths. Radiochemical assays may be used in future to confirm ages of older fish. In general, quality control at NAFC is, for the most part, lacking. There is one age reader per species and there is a recent setup of a reference collection for only yellowtail flounder; it is not used regularly. Training protocols for new agers tend to follow guidelines that have been set at other institutions.

7.6.2 *NEFSC (Jay Burnett*): Several measures to address age estimation quality are employed at the NEFSC. These include a two-reader system, in which the secondary ager reads 10-50% of samples. Percent agreement and age bias exercises are used to test the between-readers results. In addition, comparisons are made between readers of the mean lengths-at-age and associated variance (CVs) within the age estimation time series. A reference collection is currently under development, against which age-readers will be calibrated.

7.7 *Image analysis of otoliths and scales*

Several hours were used to project images of otoliths and scales from various stocks onto the screen. It was obvious that there are differences in shapes of these structures and that estimating the ages of some otoliths and scales can be difficult. However, there was good agreement among participants in many images. Several examples of annotated images from various regions are presented in Appendix VI

7.8 *General discussion and recommendations*

The ***workshop concluded*** that scales are accurate for fast growing fish and that based on the whole otolith-sectioned otolith comparisons, age validation studies should be required for older yellowtail flounder. In principle, all age estimation methods should be based on age validation. New age estimation programs in particular require age validation.

8. Discussion of Steps to a Successful Age Estimation Program.

Day 3 was used to continue discussions on the accuracy, precision, validation and quality control in aging. Many of the discussion topics are cross-referenced in Section 5 under 'Standardization of age reading protocols". The discussion also included several general conclusions and recommendations

8.1 *Developing the age estimation method*

I. *Whole otoliths vs. thin sections:* In a comparison of age readings from whole otoliths and from thin section from otoliths, the ***workshop noted*** that it doesn't know, a priori, which ages are correct, but because the thin sections are the accepted method for age estimation of many species in the literature it is assumed that ages derived from the thin section method are more accurate. After carrying out the age reading comparisons between whole and thin sections, age bias plots should be completed. Results may facilitate routine age estimation of one method, e.g. whole otoliths, up to a certain age then age estimation may have to continue using the other method, e.g. thin sections. Such comparisons should be also be carried out between ages derived from thin sections from otoliths and scales.

II. *Single structure for all stocks:* For the Grand Bank stock, it appears that thin sections are the best for older fish, but we don't have any idea of accuracy. For fast growing stocks like Georges Bank, we may be able to use any of the structures equally well. However the ***workshop noted*** that a hybrid of structures or methods for age estimation one stock could complicate the validation process. It could also be a problem with the reference collection and any exchange program. The ***workshop concluded*** that it is difficult to come to an agreement on a single structure/method across all regions.

III. *Different structures – different method for aging*: The ***workshop recommended*** that the left otolith on the side opposite the sulcus be the accepted standard. After much debate it was felt that the workshop could not recommend a standard axis in sectioned otoliths to read along. The axis chosen would depend upon the clarity (often related to the precision of the section cut and the otolith itself) in any one region. However, when exchanging otoliths, information on the axis read along should be included for each otolith. Most felt that the distal (anti-sulcus) side was never used for age determination in sectioned otoliths. For scales the ***workshop recommended*** reading along the radii in the anterior field and that cutting-over marks in the lateral fields are useful in detecting annuli.

IV. *Edge determination*: The ***workshop noted*** that age determination is based on annuli formation and edge determination, and that the translucent zone is formed in the winter-spring period. Most discrepancies in age determination between readers occur because of disagreement on edge identification. The edge seems to develop first in the dorsal and ventral area, followed by the sulcus area. The fastest growth area is generally located at the dorsal and ventral tips where the edge is likely to appear more distinct. There are software programs which remove the subjectivity in assigning the final age base on edge type. After some debate, the ***workshop concluded*** that there would be no benefit in selecting one area as a standard.

V. *Otolith reading*: For whole otolith readings, the **workshop recommended** that otoliths be soaked in glycerine/thymol solution for a couple of hours prior to reading.

VI. *Light:* The ***workshop recommended*** the use of reflected light for otoliths and transmitted light for scale impressions.

VII. *Otolith storage*: Otoliths and scales should be stored dry. Thin sections should be permanently mounted on slides or plexiglass plates (herring trays) with histoclear for long term storage. The use of Krylon as a mounting medium was not recommended because it yellows over time. Permount should be used instead .

VIII. *Prior knowledge about the otolith*: The ***workshop concluded*** that having fish length, sex, area, and time of capture available during reading was more helpful rather than being detrimental to the age reading.

IX. *Otolith weight*: For faster growing fish the ***workshop noted*** that the relationship between weight of an otolith and either the age and/or the length of the fish should be investigated. Otolith weight frequencies often give better separation of modes than fish length. Integrated software such as MIX would help to do this separation with approximately 50-100 otoliths being used. Otolith shape analysis hasn't shown much promise but was useful for some stock identification and growth rates study.

X. *First annulus:* The ***workshop recommended*** that the following methods be considered: 1) determining the microstructure of daily increments, 2) using modal analysis of length frequency data, and 3) measuring the width of the first annulus for future separation.

8.2 *Age validation*

I. The ***workshop concluded*** that marginal increment analysis of older fish offers very little promise in age studies of yellowtail flounder on the Grand Bank. Modal analysis of length frequency data was shown to have some use as an age validation technique. Following modal peaks of strong year-classes from research vessel surveys in Grand Bank yellowtail flounder seem to agree with modal peaks in ages 1-4. For older fish, age validation should consider age estimation information from recaptured tagged fish and also bomb radiocarbon assays. The ***workshop recommended*** that a validation study be carried out for the older age groups.

II. For the Georges Bank stock, the ***workshop recommended*** that a validation study be carried out for the older age groups.

8.3 *Preparation of reference collection*

Purpose of the reference collection: the collection is used for calibration of in-house reader consistency, monitoring of drift and training of new age readers. It should consist of otoliths or scales that were read by multi-readers from an exchange to arrive at the most appropriate age and validated ages. The ***workshop recommended*** that all regions set up a reference collection.

8.3.1 *Selection criteria and monitoring*

The reference collection should be representative of various categories of otoliths (difficulty, age, length, sex, seasons, gear, area). The collection should be randomly selected, spread out over a range of ages and large enough to minimize memorization. The ***workshop recommended*** that a minimum of 500 otoliths or scales be used for the reference collection. This collection should contain validated ages, if available, and ages reached by consensus by multiple readers. The collection must contain samples from both research and commercial data to capture the essence of differences in growth rates. Samples should be spread across several years.

8.3.2 *Training*

The ***workshop recommended*** a minimum of 100 otoliths or scales from the reference collection be used for the training session.

8.3.3 *Exchange program*

The ***workshop recommended*** that to achieve consensus ages, each region should circulate a collection of 200 otoliths (thin sections should be mounted on plexiglass) or scales from its reference collection to each institute. The collection should consist of validated age samples if available. This would be a one time only event. Consensus ages would then be used to test for drift. If validation cannot be done on the otolith method (true age of the fish is in doubt), consensus ages can be used. If experts disagree on the age, the median age of all experts can be used (x-axis for age bias plot).

Alternatively, the ***workshop concluded*** that digital images could be circulated instead of the structures themselves. A CD writer was recommended as the most appropriate medium to contain these images. Protocols for annotating each image should include information on length, sex, collection date, axis read, where edge read and stock area. The workshop concluded that a reliability check for each image was unnecessary, however, it noted that a “comment block” should be used on each image to note good or bad otolith. The ***workshop concluded*** that an inter-regional reference collection was not feasible.

8.4 *Quality Control*

Frequency of monitoring the reference collection: How often should the reference collection be read? Some ideas that were brought up: once before production age estimation, once before and once after production age estimation, or during production age estimation (e.g. Isabelle Forrest at GFC reads her collection after every 1000 production otoliths). The **workshop recommended** that before production age estimation begins, the following sequence should take place:

Step1, perform a pre-calibration test by reading ~100 otoliths from last years collection to ‘warm up’ and then use age bias plots and CV’s to test for drift.

Step 2, begin production age estimation

Step 3, re-read 100 otoliths from the reference collection and 100 otoliths randomly chosen from current year's otoliths. Test for drift with age bias plots and CV's.

Note: The responsibility for selection of otoliths in Step 3 is that of the supervisor or another independent entity. The frequency of calibration may be once to twice a year in most regions.

8.4.1 *Bias and precision*

The ***workshop recommended*** that age bias plots and CV's were the most acceptable statistics to evaluate the results of monitoring, i.e. within reader drift and variability in age determination. How much bias is too much? To look for bias, a trend or tendency should be noted in terms of a deviation from a 1:1 line. A deviation of one year might be important. Also important is to look for bias in young fish or in abundant age classes. Based on past experience bias is mostly observed in young and old fish.

Which CV reference level should be used to identify the threshold between acceptable and unacceptable degrees of precision among yellowtail age readers? No one CV level is acceptable for all species and it is stock and structure specific. Precision is independent of age and within reader precision will be better than between reader precision. In the literature (Campana 2001) CV's of 0-25% in annual age estimation are cited with the majority being less than 8%, (modal precision in the 4-5% range). However, until there is a build up of repeated testing in each region the ***workshop concluded*** that it is difficult to set a target reference level.

If there is bias or the reader is unable to attain an acceptable level of precision, how should age readers attempt to fix this problem? Using age bias plots can help to determine where the bias is located. The ***workshop endorsed*** the Beanlands (1997) Inter-Regional Ageing Workshop recommendations: 1) read the training section of reference collection to recalibrate; 2) conduct a blind test with the reference collection (test portion) and run appropriate tests, 3) re-read all otoliths from the point where the bias was first detected.

It has to be remembered that new agers start at a high CV and work their way down. When monitoring a reader, the mean length and variation at age can be an indication of a problem.

8.4.2 *Updating the reference collection*

How often and when? First, it is not desirable to continually replace otoliths in the reference collection because over time, the reference collection will get more recent and will not detect drift. After using the 100 otoliths from the current year in the monitoring phase (step 3) add these to reference collection. In the case of otoliths stored in glycerine and used for the reference collection digital images should be taken.

8.4.3 *Secondary age readers*

The ***workshop concluded*** that a second age reader is not necessary for Quality Control. It stretches the resources, since one needs to wait for the second reader's verification and it is not clear what is the advantage re-reading the otoliths of the primary ager. However, where a second age reader is used ***the workshop concluded*** that in carrying out statistical analysis of age determination, percent agreement was not an appropriate method to capture a measure of variability in age agreement between readers. The ***workshop recommended*** that age bias plots and CVs be the standard method.

9. Revision of historical age readings (Archival conversions).

Based on the whole vs sectioned otolith comparison, yellowtail flounder above 35 cm appear at risk of being under-aged in the Grand Bank collection. This may be the case for other stocks as well. If we assume sectioned otoliths give an age close to the true value then older ages will have to be re-aged. This will be costly but necessary for an age based analytical assessment. The **workshop noted** that sub-sampling of otoliths stratified by length, sex, area and season could be tried. If you are not confident in the sub-sampling then the entire collection will have to be re-read as was the case for Scotia-Fundy haddock.

10. Summary of recommendations

I. Age Reading Protocols

1. The ***workshop recommended*** that the left otolith on the side opposite the sulcus be the accepted standard. For scales, the ***workshop recommended*** reading along the radii in the anterior field.

2. The ***workshop recommended*** that otoliths be stored dry and not in glycerine which may cause deterioration.

3. The ***workshop recommended*** that whole otolith be soaked in glycerine/thymol solution for a couple of hours prior to reading.

4. The ***workshop recommended*** reflected light for otoliths and transmitted light for scale impressions.

5. *Determination of first annulus:* The ***workshop recommended*** that the following methods be considered: 1) determining the microstructure of daily increments, 2) using modal analysis of length frequency data, and 3) measuring the width of the first annulus for future separation.

II. Specific stocks

❖ The ***workshop recommended*** that age readers from the NEFSC should examine samples of otolith thin-sections from the Canadian fishery on Georges Bank for comparisons with ages determined by Canadian age readers.

❖ For the Georges Bank and the Grand Bank stocks, the ***workshop recommended*** that a validation study be carried out for the older age groups.

III. Statistics

The ***workshop recommended*** that age bias plots and CV's were the most acceptable statistics to evaluate the results of monitoring, i.e. within reader drift and variability in age determination. The ***workshop concluded*** that percent agreement was not an appropriate statistical method to capture a measure of variability in age agreement between readers.

IV. Reference Collection

- The ***workshop recommended*** that all regions set up a reference collection for calibration of in-house reader consistency, monitoring of drift and training of new age readers.

- The ***workshop recommended*** that a minimum of 500 otoliths or scales be used for the reference collection.

- The ***workshop recommended*** a minimum of 100 otoliths or scales be used for the training session.

- The ***workshop recommended*** that to achieve consensus ages, each region should circulate a collection of 200 otoliths (thin sections should be mounted on plexiglass) or scales from its reference collection to each institute.

V. Calibration and production aging

The ***workshop recommended*** that before production age estimation begins, the following sequence should take place:

Step1, perform a pre-calibration test by reading ~20 otoliths from last years collection to 'warm up' and then use age bias plots and CV's to test for drift.

Step 2, begin production age estimation.

Step 3, re-read 100 otoliths from the reference collection and 100 otoliths randomly chosen from current year's collection. Test for drift with age bias plots and CV's.

Note: The responsibility for selection of otoliths in Step 3 is that of the supervisor or another independent entity. The frequency of calibration should be once to twice a year in most regions.

11. Conclusions

Slow growing cold-water species are often underaged when using the whole otolith method and investigation into sectioned otoliths should be pursued. A comparative examination should be carried out in the manner described in this workshop.

12. Future recommendations

1. Progress on implementation of above recommendations should be reviewed in 3 years.

2. OTC (oxytetracycline) marking during regular tagging studies should be encouraged. At same time take a scrape of scales for age estimation.

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Appendix l

Agenda for the Canada-United States Yellowtail Flounder Age Reading Workshop, November 28-30, 2000
The Chainlocker Room at The Battery Hotel and Suites, 100 Signal Hill Road
St. John's, Newfoundland A1A 1B3

Terms of Reference:

- Evaluate the various structures and methods used to age yellowtail flounder in various regions;
- Develop standardized protocols for age estimation, training, testing and consistency of evaluation;
- Establish a mechanism for inter-lab exchange of age estimation material; and
- Record workshop proceedings and report to NAFO Scientific Council in June 2001.

Tuesday, November 28th

Convener: *Steve Walsh* *Rapporteur: Rob Perry*

<u>0900 –1700 hrs</u>**:**

1. Introduction of participants and review of Term of References of the workshop.

2. Presentation by each institute of current age estimation methodologies. The focus should be on distribution, age and growth, maturity, age validation studies and general protocols used in age estimation yellowtail flounder. (Steve W., Jay, Jeremy, Heath, Isabelle and Mark) (see attachment #1 for details).

3. Discussion of standardization of terminology (see attachment # 2) (Jay to lead).

4. Discussion of standardization of age reading protocols (see attachment # 3) (Jay to lead).

5. Slide show on scales and otoliths – discussion topics (Jay)
    a) Nucleus and first annulus
    b) Best axis to count along
    c) Zone identification
    d) Checks -split zones, split annuli, and false annuli.
    e) Edge growth
    f) Stock differences in structures.

6. Hands on age estimation of representative sampling of otoliths from each region to get a feel for differences (Jay to lead).

7. Start outline of workshop report (Steve W.).

8. Demonstration of equipment and techniques for age estimation yellowtail flounder.

Wednesday, November 29th

Convener: *Jay Burnett* *Rapporteur:* *Heath Stone*

<u>0900 –1700 hrs</u>**:**

1. Continuation of any unfinished discussion of Day 1 presentations.

2. Reading Comparisons

    Presentation and discussion of results of otolith versus scales readings carried out by NEFSC, SABS, NAFC (Jay, Karen and Heath).

3. Introduction to determining accuracy, precision, age validation and quality control in otolith age estimation (Steve Campana).

4. Presentation by those labs ageing yellowtail flounder on studies related to Topic #3 (see Attachment # 4) (Jay, Karen and Rob).

5. General discussion on current practices in accuracy, precision, age validation and quality control in otolith age estimation (Steve C.).

6. Continue outline of workshop report (Steve W.).

7. Further demonstration of otolith preparation techniques if time is available.

Thursday, November 30th

Convener: Steve Campana *Rapporteur: Karen Whalen*

<u>0900 –1500 hrs</u>**:**

1. Continuation of any unfinished discussion of Day 2 presentations. Selection criteria and protocols for establishing reference collection and training protocols for new readers. This will include discussion of the problem of single age readers and the use of image archiving material.

2. Discussion on establishing and setting of protocols for an otolith/scale exchange program.

3. Discussion of archival conversions (deriving conversion factors for correcting historical ages).

4. Discussion and recommendations regarding the terms of reference (Steve W. Steve C. and Jay).

5. Presentation of draft outline of the workshop report (Steve W.).

Appendix II - Regional Reports on Biology and Age Reading of Yellowtail Flounder
Yellowtail Flounder Research on the Grand Bank

by

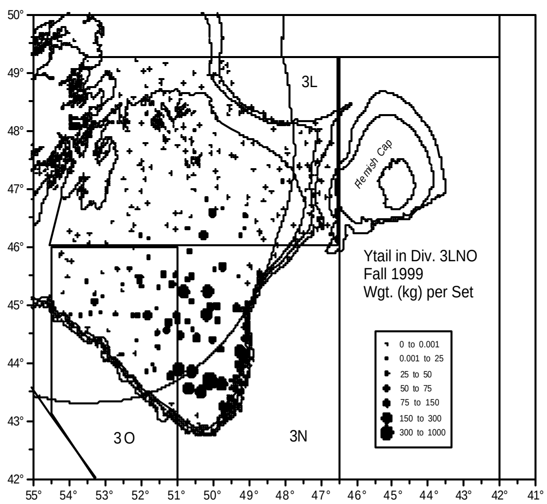
Stephen J. Walsh, Mick Veitch & Karen Dwyer
Northwest Atlantic Fisheries Center, St. John's

Introduction

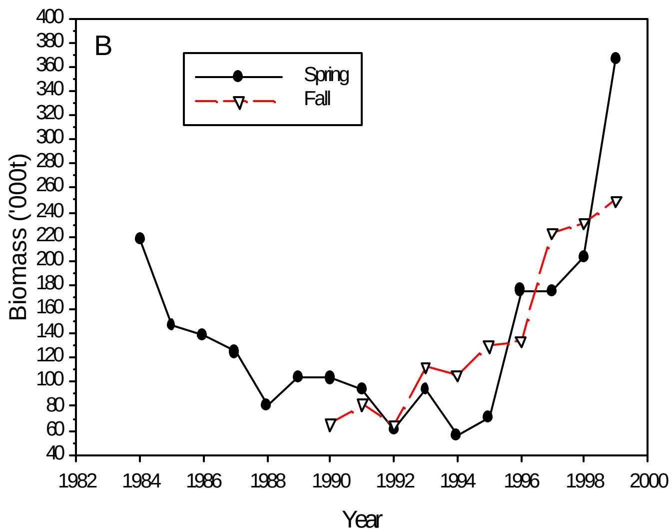
The Grand Bank stock, Northwest Atlantic Fisheries Organization (NAFO) Division 3LNO, is under quota regulation and because it straddles Canada's 200 mile limit it is managed by NAFO. NAFO's Scientific Council meets annually to assess the size of this resource and several other finfish and shellfish species. An international fishery for yellowtail flounder on the Grand Bank developed in the 1960s and grew to yield a peak of 39,000 t in 1972. Although the earlier fishery was prosecuted by many Canadian and non-Canadian trawler fleets, the present day fishery is prosecuted mainly by Canada inside the 200 mile zone and by European Union fleets in NAFO Regulatory Area of Division 3N, known as the Tail of the Bank.

Since 1973, the stock has been managed by regulating a total allowable catch (TAC), ranging from 7000 tons to 50,000 tons. Over the past three decades the stock had shown large fluctuations in biomass levels. After a continuous decline in survey biomass and poor recruitment during the late 1980s and early 1990s a fishery moratorium was imposed by the NAFO Fishery Commission in 1994. The fishery re-opened in 1998 with a precautionary TAC of 4,000 tons.

Distribution.

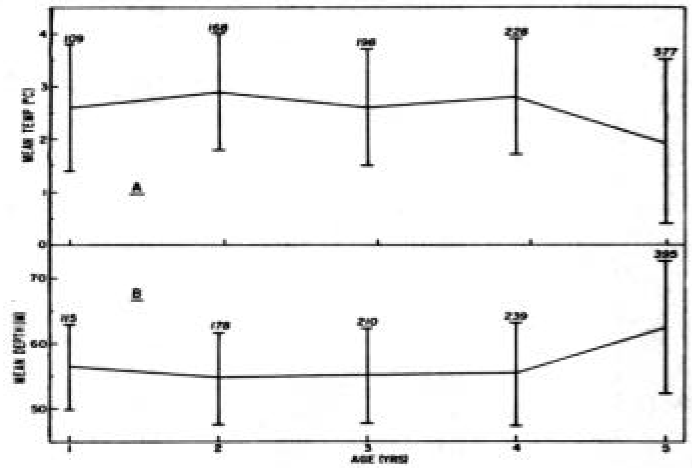


Biomass



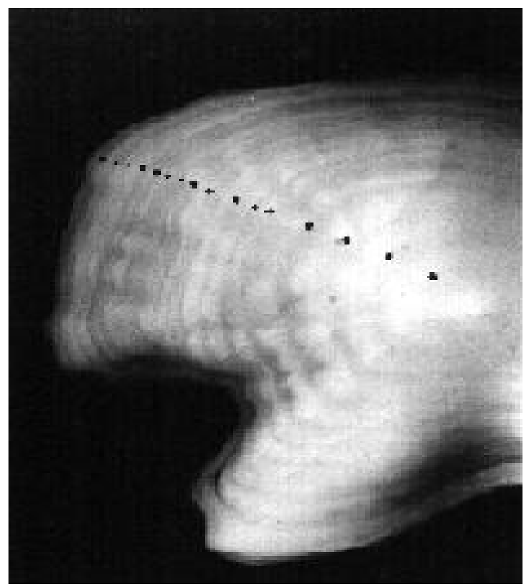
Factors affecting distribution

- Temperature range -1.2 to 5.8 ^{0}C
- Depth range 40-100 m
- Sandy bottom
- Spawning May to Sept



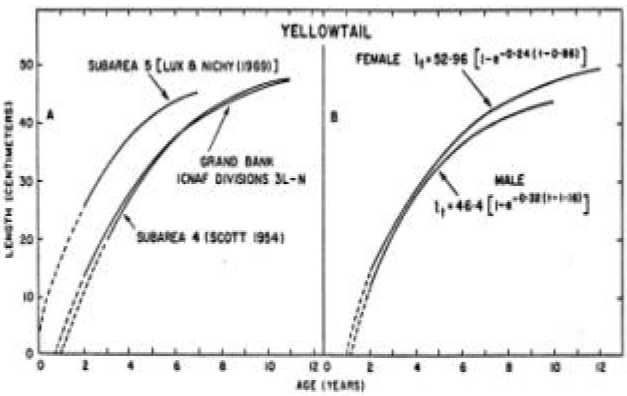
Age Sampling Protocols

- Sampling since 1949
- North GB 5/2cm/sex
- South GB 3/2cm/sex
- Stored dry in envelopes
- Read whole-Grind older samples
- No distinction between left or right otolith
- Edge recorded- not used
- Birthday is January 1



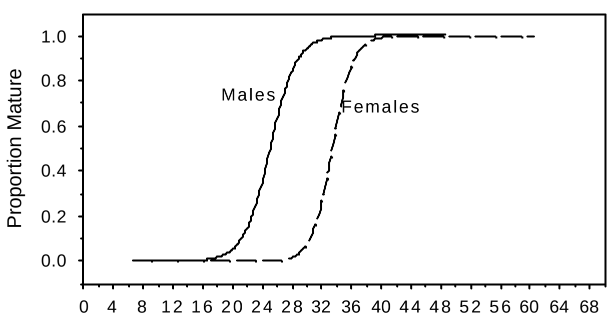
Growth

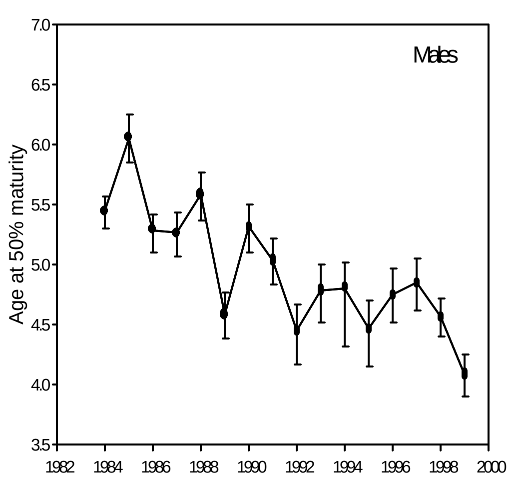
Historic view of growth from Pitt (1974)

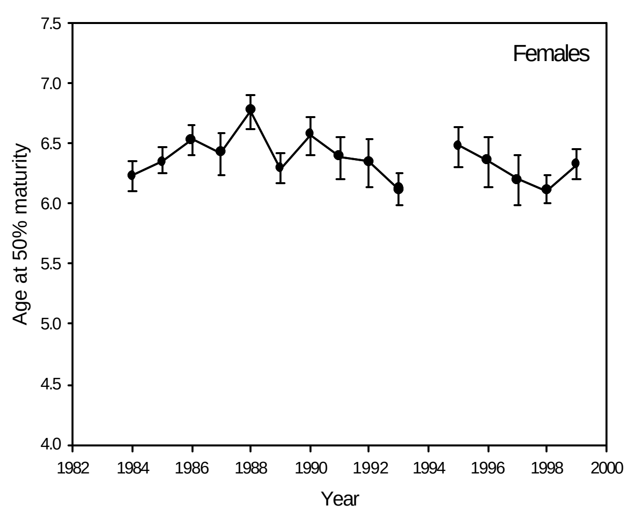


<u>Maturity</u>

On the Grand Bank, males mature at an average age of 4 years (25 cm) and females mature at an average age of 6 years (34 cm).

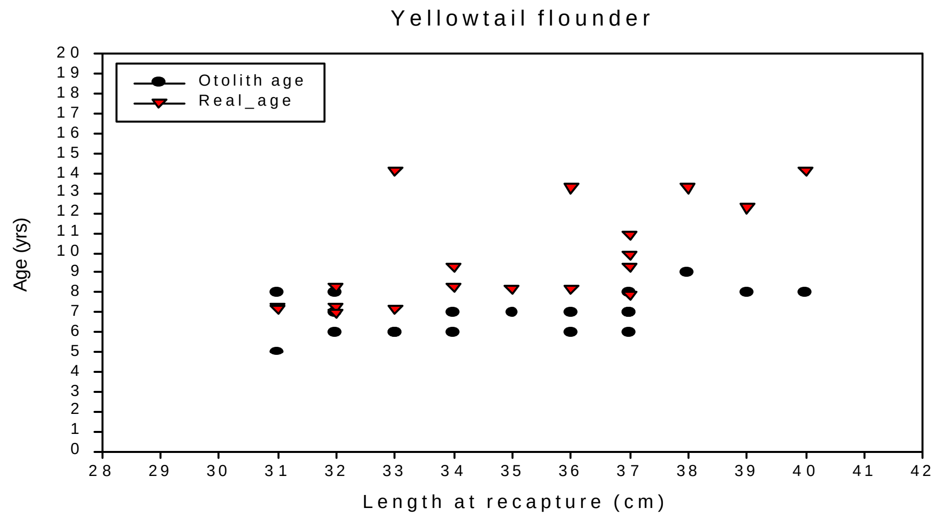




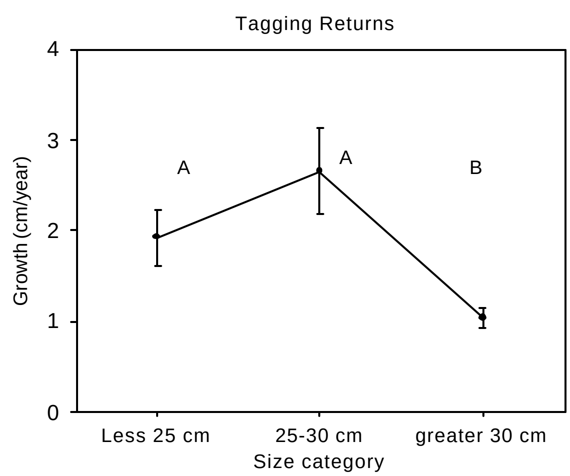


<u>Tagging Studies - information on age and growth</u>

During the 1990-1993 period tagging studies of juvenile yellowtail flounder on the southern Grand Bank took place. The fishery was under moratorium from 1994-1998 and since it re-opened several tags have been returned



- Fish were found to be older than we were ageing them
- Fish were found to be growing much slower than we had estimated



Tagging returns indicate that yellowtail flounder are older than we are ageing them and growing slower than we estimated.

Fresh approach to investigating age and growth of yellowtail flounder, 1999-onward

Old system

- Research otoliths 1949-present
- Commercial otoliths 1965-present
- one age reader
- underestimating older ages
- underestimating growth???

New Approach for 2000

- Two age readers
- Sectioning, staining and baking otoliths
- Reading scales
- Ages from length frequency
- Larval length frequencies
- Reference collection
- Age reading workshop

References

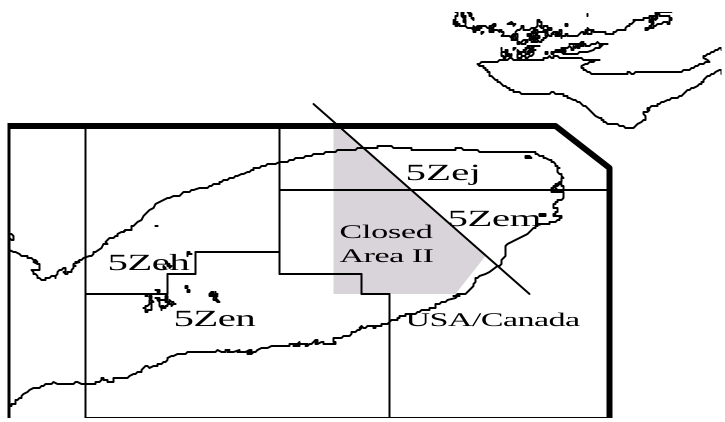
Pitt, T.K, 1974. Age composition and growth of yellowtail flounder (Limanda ferruginea) from the Grand Banks. J. Res. Fish. Bd. Can. 31:1800-1802

Georges Bank Yellowtail Flounder

By

Heath Stone and Peter Perley
St. Andrews Biological Station, St. Andrews

- Yellowtail flounder on Georges Bank are considered to be a discrete stock and are part of a larger population which includes other stocks off Cape Cod and southern New England.
- Fished by Canadian and US fleets using bottom trawl gear, has a long history of exploitation by US fleet (back to 1930's).
- Currently managed as a transboundary resource, with 5Zhjmn as the management unit.
- Canada and USA share information on commercial catches and research surveys to evaluate the status of this stock.
- Note closed area (referred to as Closed Area II) adjacent to the International Boundary (has contributed to stock rebuilding).



Canadian Spring 2000 Survey Distribution of Yellowtail Flounder

- Canadian surveys show that resource distribution is mainly in 5Zm and that the range has been expanding in recent years.
- Common at depths between 37- 91 m on sandy substrates on NE portion of bank.
- Stock was overexploited in early 1990's, but has been rebuilding rapidly over past 5 years.
- Biomass is now approaching MSY and there are several strong year classes present.

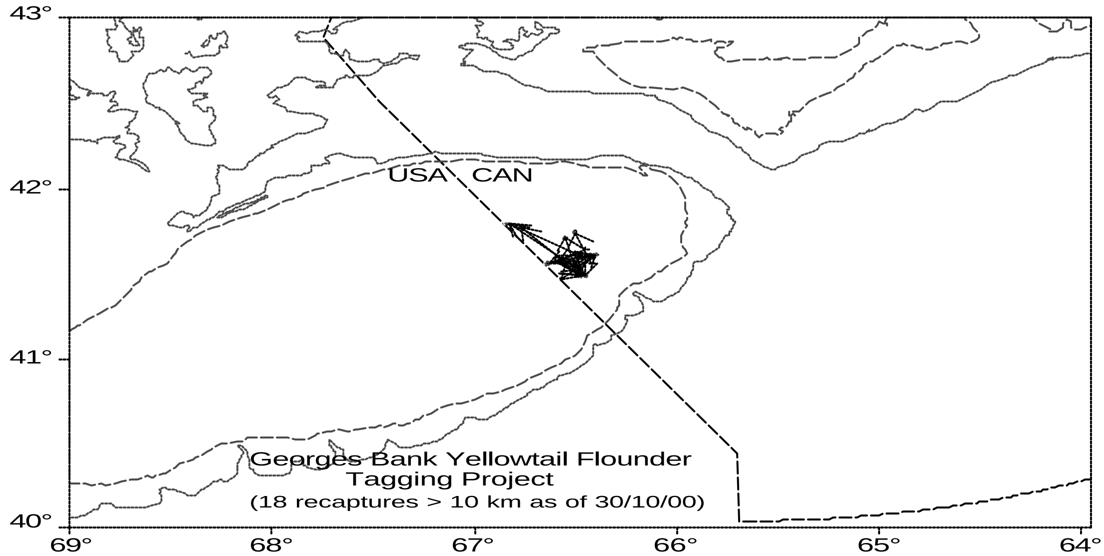
Size composition

- Overall size composition represented by fish in the 29-47 cm range (broader than commercial catches) with females more abundant at sizes > 38 cm.
- Mean size of males and females has also increased over past 5 years.
- In some years, modes are apparent for smaller males and females, (might be of use to validate of ages 1-2), but it is difficult to track modal progressions from year to year.

Yellowtail Flounder Tagging

1. 2,165 yellowtail flounder tagged and released on Georges Bank in the "Yellowtail Hole" (December, 1999).

2. 84 recaptures as of November, 2000 (4% return rate).
3. Average distance between release and recapture sites was 8 km (range: 0-39 km).
4. 18 recaptures at distances > 10 km, with an overall vector of movement to the northwest.
5. Findings support results of earlier studies which suggest that yellowtail flounder are quite sedentary and do not move far.
6. Hope to continue tagging in 2001, with possibility of double tagging and scale removal on some fish to assist in age validation (if recaptured).



Maturity (based on Royce et al. (1959)

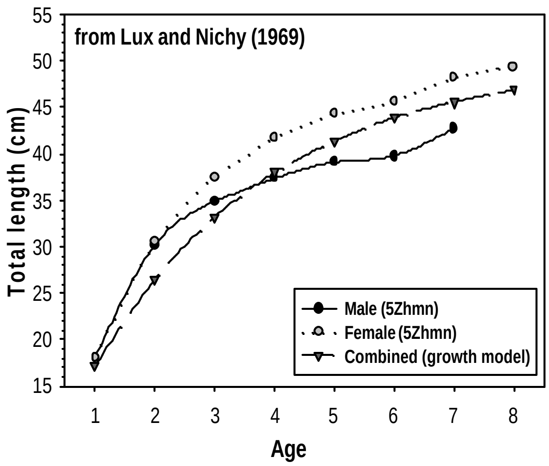
Females: 50% mature at 32cm TL, 90% at 40cm TL
52% mature at age 2, 67% at age 3, 100% at age 4

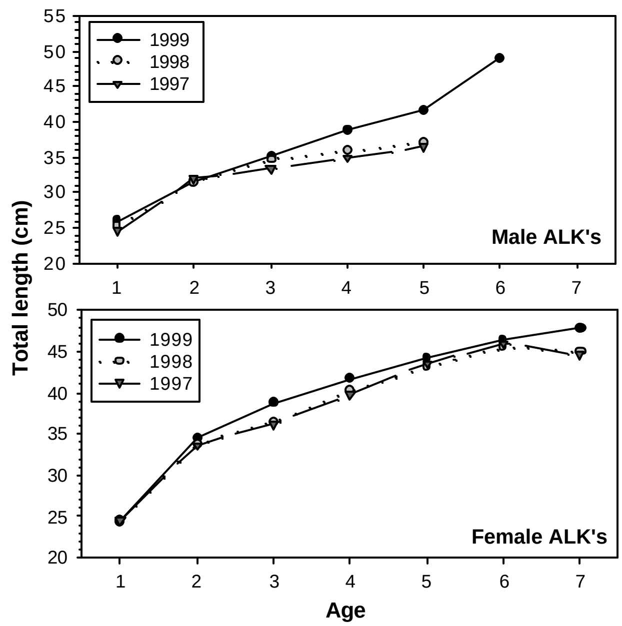
Males: most mature at 26 cm TL
84% mature at age 2, 92% at age 3, 100% at age 4

- Spawning on Georges Bank occurs in second quarter, peaking in May.
- Projections for stock assessment assume 100% are mature at age 3 (both sexes).

Age and Growth

- Most detailed study on Age and Growth of GB yellowtail was conducted by **Lux and Nichy (1969)** using scales.
- Good documentation of first annulus formation and growth through age two.
- Partial validation of aging for fish aged 2-5 was achieved through recapture of 35 fish that had been sampled for scales.
- Confirmed that growth is **sexually dimorphic**, females grow faster and reach larger sizes than males, and that length at age by sex starts to differ after age 2.
- Few old fish (6+) were actually examined in this paper but authors concluded that age assessment of older fish was difficult because of “narrowing of the growth zones” and frequently disagreed on ages for fish over age 5.





- Age length keys for separate sexes from USA Fall survey, port and at sea samples are currently applied to Canadian catches.
- Mean lengths at age from ALK's used in past three stock assessments are generally similar to those calculated by Lux and Nichy.
- Provides reassurance that aging has been fairly consistent, although there is some annual variability in length at age, especially for males and at older ages (6+) for both sexes.

<u>SUMMARY</u>

Trans-boundary Resources Assessment Committee has recommended that a Canadian age estimation program for yellowtail be developed at SABS, which is now our primary objective.

Several Issues to Address:

- The **Lux and Nichy** paper is of considerable assistance for interpretation of annuli on scales up to age 5. However, it is of very limited help for older fish. This is of concern for the GB stock, since it has been subject to low rates of exploitation for several years now, and older ages are expected to become an important component of the overall catch.
- For USA and CDN fisheries, ages 2-4 dominate the catch but we do not see many older fish in the catch even though average size has increased over the past 5 years.
- It is difficult to detect and track strong/weak year-classes through time.
- Weight at age shows a rapid decline from 1989-1990 and an increase from 1999-2000 (Change in age reader? Spatial/temporal variability in sampling? Change in growth rates?).

Aging Methodology and Protocols

- Currently we are in the process of examining otoliths, scales and otolith sections
- Not clear which structure is best to use for age determination
- Hope to have a better idea at the end of this workshop

Sampling and Storage: RV Survey Collections

- Inventory at SABS includes over 5000 otolith pairs from Canadian RV survey collections (1987-1991; 1996-2000).
- Length frequencies from each set are sub-sampled for age material: 1 set of otoliths for each 1 cm grouping by sex.
- Stored in vials containing 70% glycerine and water solution with 0.9 gm/l thymol added to limit bacterial activity.

Year	Cruise	No. otoliths by sex			Total
		unid	male	female	
1987	N77	0	107	114	**221**
1988	N97	0	158	155	**313**
1989	N116	0	151	95	**246**
1990	N133	1	281	244	**526**
1991	N148	1	286	190	**477**
1992	N165	-	-	-	**0**
1993	T134	-	-	-	**0**
1994	N200	-	-	-	**0**
1995	N216	-	-	-	**0**
1996	N237	0	335	272	**607**
1997	N254	7	409	431	**847**
1998	N773	5	261	245	**511**
1999	N871	4	376	404	**784**
2000	N965	1	582	557	**1140**
Total	**all**	**19**	**2946**	**2707**	**5672**

Sampling and Storage: Port Sample Collections

- Over 3,000 otolith pairs by sex available from Canadian port samples.
- For each sample, up to 230 yellowtail per trip are measured and sexed; otoliths are extracted from a subsample 30 fish.
- Stored dry in envelopes and later transferred to vials containing a glycerine/thymol solution.
- Scale samples were taken during the 2000 fishery for comparisons with otolith age determinations.

Year	No. of samples	No. otoliths by Sex		Total
		male	female	
1977	1	-	-	**40**
1978	1	-	-	**21**
1982	5	-	-	**107**
1994	3	34	46	**80**
1995	8	78	107	**185**
1996	8	73	111	**184**
1997	11	109	162	**271**
1998	17	194	278	**472**
1999	21	217	375	**592**
*2000	39	449	691	**1114**
Total	**114**	**1154**	**1770**	**3066**

*** as of October. 2000**

Note: To date, very few age determinations have been made from RV survey and port sample collections

Equipment Available at SABS

- Optimus Image Analysis System and Software (V 6.5)
- Binocular microscopes
- Jeweler's press (scales)
- Scale projector
- Otolith sectioning machine (Hebert surface grinder adapted to accommodate a diamond-edged wheel)
- Belt and disc sander

Preparation Techniques and Reading Protocols

- Use information on fish length and sex as a guide.
- Assume Feb. 1 as birthday (translucent outer edge counted as an annulus).
- **Whole otolith pairs** are examined in glycerine/water solution under reflected light in a watch glass; read along the rostrum on distal surface.
- **Scale impressions** on laminated plastic (as per method of Penttila) are examined under transmitted light; first annulus very small (i.e. Lux and Nichy (1969); Penttila (1988)).
- **Transverse sections of otoliths** (0.8 mm thickness) in polyester resin are examined in glycerine under reflected light; read along the edge of sulcus or laterally along section.
- Use 10-15x magnification on all structures.

References

Lux, F.E. and F.E. Nichy. 1969. Growth of yellowtail flounder, *Limanda ferruginea* (Storer) on three New England fishing grounds. ICNAF Res. Bull. 6: 5-25.

Penttila, J. 1988. Chapter 19: Yellowtail flounder *Limanda ferruginea*. *In*: Penttila, J. and L.M. Dery (eds.), Age determination methods for Northwest Atlantic species. NOAA Tech. Rpt. NMFS 72: 119-124.

Royce, W.F., RF. Buller, and E.D. Premetz. 1959. Decline of the yellowtail flounder (*Limanda ferruginea*) off New England. Fish. Bull. 146: 1-267.

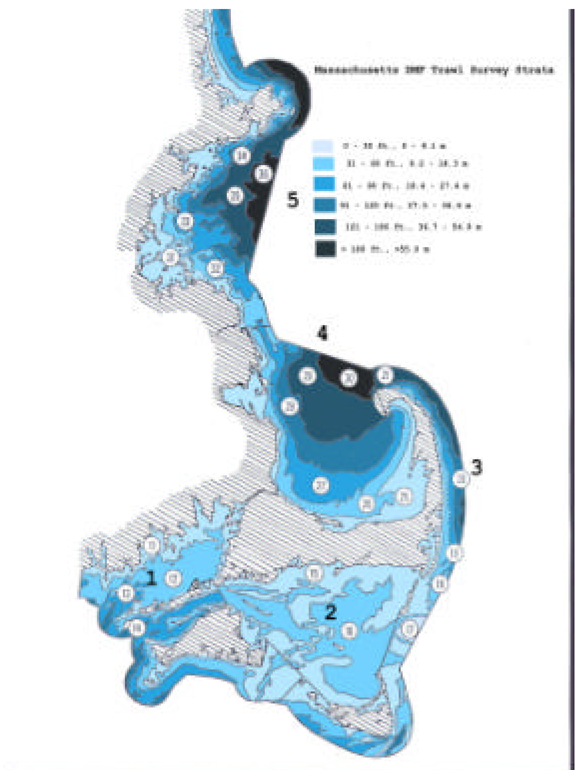
Sampling of Cape Cod Stock Yellowtail Flounder by the State of Massachusetts Trawl Survey.

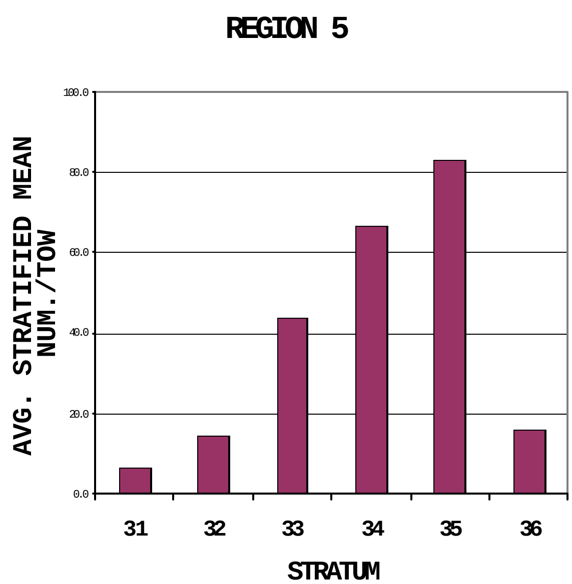
by

Jeremy King
Massachusetts Division of Marine Fisheries, Pocasset

Massachusetts Division of Marine Fisheries (MDMF) has conducted a bottom trawl survey in state territorial waters every May and September since 1978. The MDMF survey regions 3–5 (including East of Cape Cod, Cape Cod Bay, Massachusetts Bay and north to the New Hampshire border) while not encompassing some of the prime fishing grounds of Cape Cod stock yellowtail flounder (i.e. Stellwagen Bank and deeper water east of Cape Cod) represent the coastal portion of the stock. The Massachusetts survey fishes with a ¾ size North Atlantic type two seam otter trawl (11.9m headrope – 15.5m footrope) rigged with a 19.2m chain sweep with 7.6cm rubber discs.

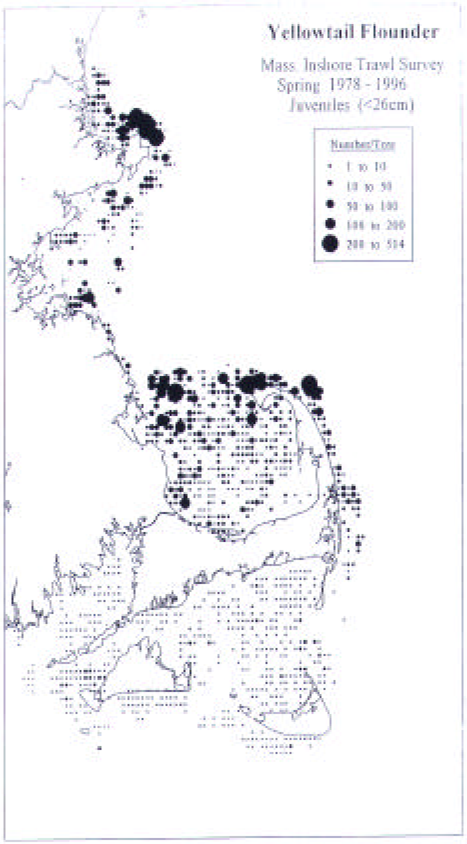
On average, greater than 1600 yellowtail are captured each spring and yellowtail are present at greater than 40 stations. In autumn, greater than 1000 yellowtail are captured at more than 30 stations. The depth stratum 36.7m – 54.9m produces the greatest yellowtail catches in each of the three survey regions.



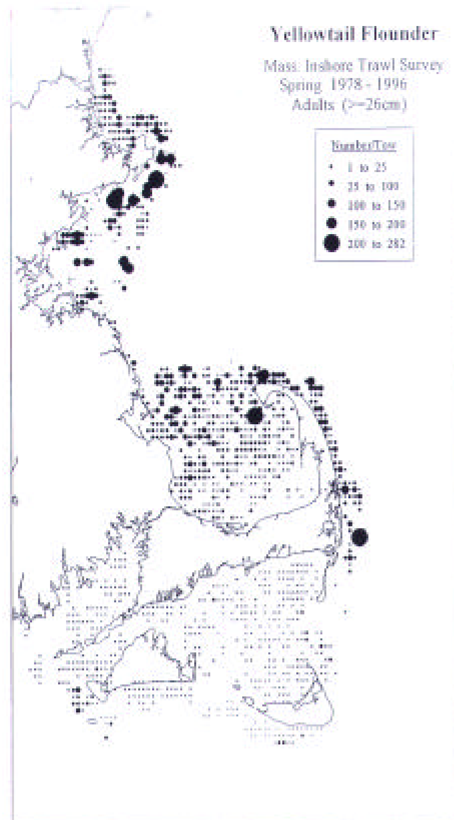


Stratum 35 (36.7m – 54.9m) in Region 5 (Mass. Bay and north to the New Hampshire Border) is not only one of the highest producing strata, but is also the greatest stratum in total area within regions 3-5 and thus, is the most influential in Cape Cod stock yellowtail population metrics produced from the Massachusetts survey.

Juvenile catch distributions change little in the survey area between spring and autumn except in Cape Cod Bay where juveniles appear to redistribute from outer Cape Cod Bay in spring to the shores of the bay in autumn. Adults in the vicinity of Cape Anne appear to redistribute from South of Cape Anne in spring to the north in autumn. The largest catches of juveniles occurred at 4-8ºC in spring and 8–11ºC in autumn, while adult temperature preferences appeared similar with greatest spring catches at 4-9ºC and 8–11ºC in autumn (Johnson, et al. 1999).

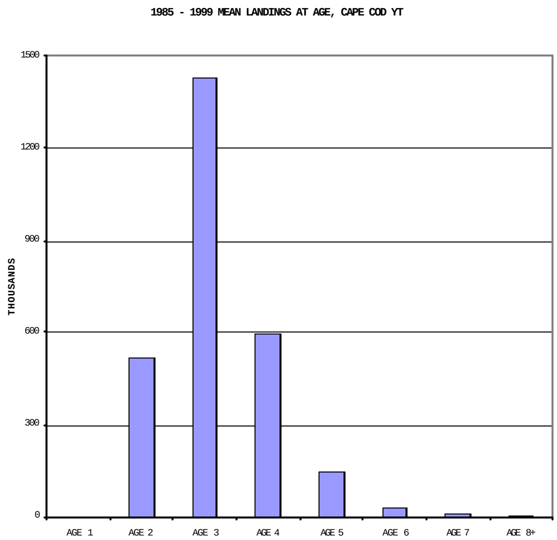


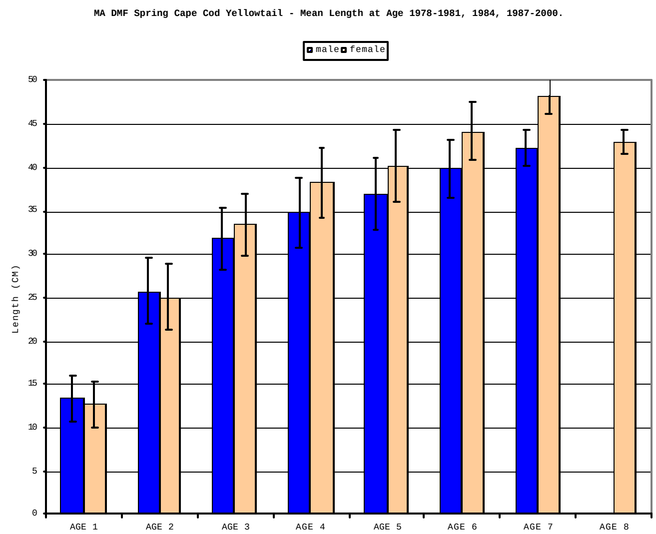
Distribution and abundance of juvenile yellowtail flounder from Massachusetts Inshore Spring research trawl surveys, 1978 - 1996.



Distribution and abundance of adult yellowtail flounder from Massachusetts Inshore Spring research trawl surveys, 1978 - 1996.

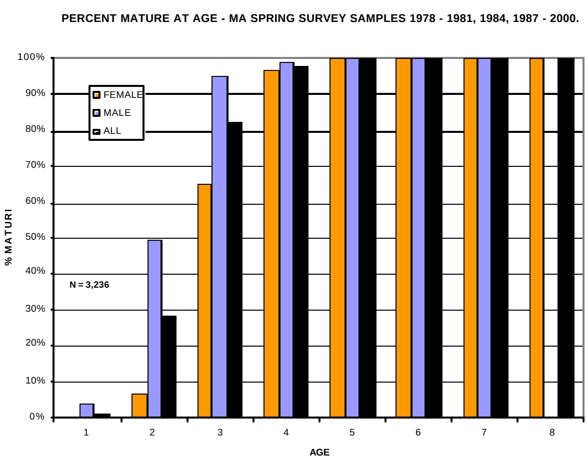
Recent MDMF efforts to differentiate yellowtail survey catches by sex reveal that the sex distribution in the Massachusetts survey area differs from 50:50. A skewed sex distribution is most evident in the spring in stratum 35. In spring 2000, 67% of the yellowtail catch in stratum 35 were males.

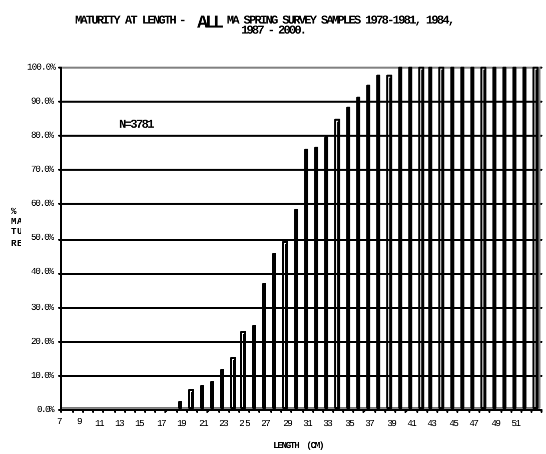


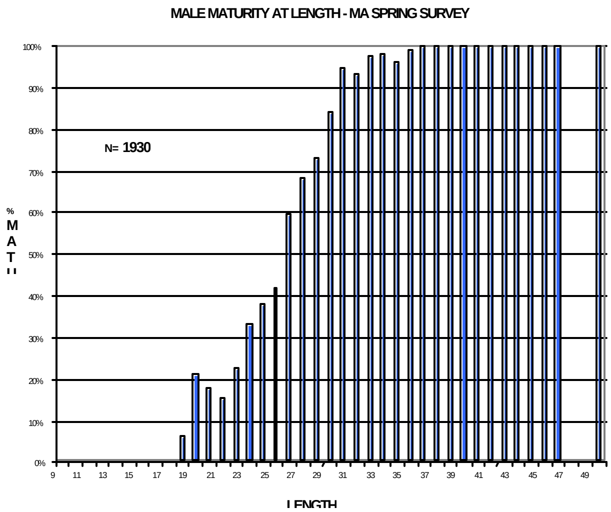


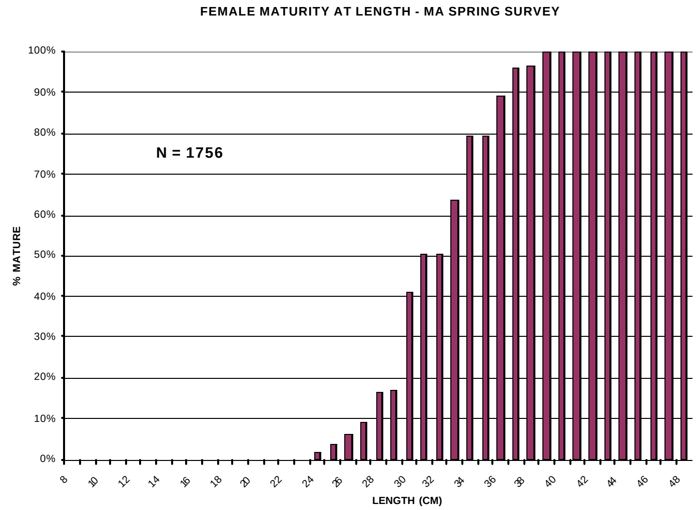
Including surveys completed this year, 4,836 age samples have been collected on spring surveys and 2,599 samples collected in the autumn. A subset of age samples from 20 spring cruises indicates that fewer than 5% of samples collected represent fish older than age 4. VPA results 1985-1999 indicate that the commercial catch is mostly (93%) composed of ages 2 – 4 (Cadrin, 2000).

Average length at age, as well as maturity at age and length, are reported for a summary of 19 of 23 spring cruises. Females are larger at age than males beginning at age 3, while males mature at a younger age and smaller size than females.









References:

Cadrin, S.X. 2000. Cape Cod yellowtail Flounder. *In* Assessment of 11 Northeast Groundfish Stocks through 1999. NEFSC Ref. Doc. 00-05: 83-98.

Johnson, D.L., Morse, W.W., Berrien, P.L., and J.J. Vitaliano. 1999. Essential Fish Habitat Source Document: Yellowtail Flounder, *Limanda ferruginea*, Life History and Habitat Characteristics. NOAA Technical Memorandum NMFS-NE-140. 29 p.

Biology and Age Estimation of Yellowtail Flounder in NAFO Subareas 5 and 6

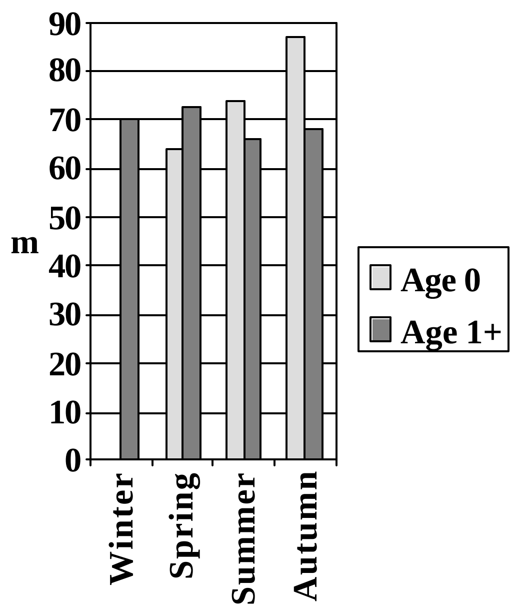
by

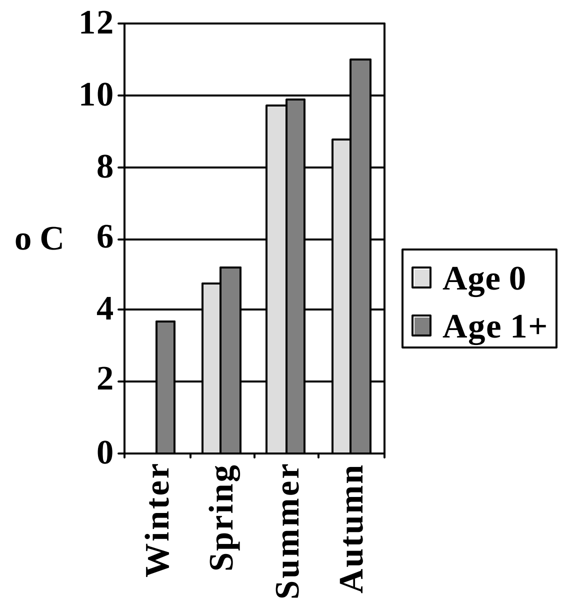
Jay Burnett and Vaughn Silva
Fishery Biology Program, Northeast Fisheries Science Center, NMFS
Woods Hole, Massachusetts

Yellowtail Flounder Stock Structure in Subareas 5 and 6

- **Mid-Atlantic (Subarea 6)**
- **Southern New England (5Zw, west of 69o)**
- **Georges Bank (5Ze, east of 69o)**
- **Cape Cod**
- **Gulf of Maine (5Y)**

Distribution by Depth



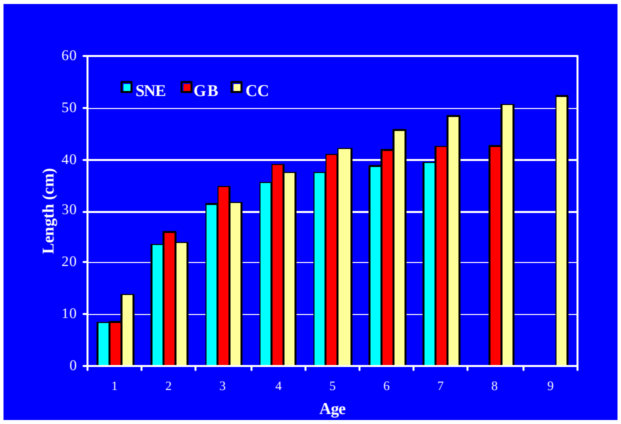


- 20-40 fathoms (37-73 m)
- 5-95 f (9-174 m)
- Sand or sand/mud bottom
- Very little seasonal movement
- Murawski and Finn 1988

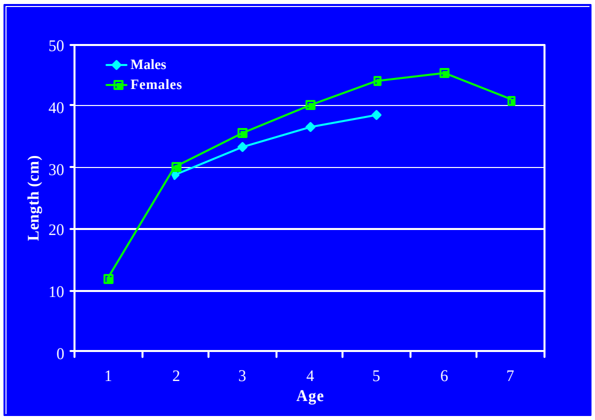
Distribution by Temperature

- 2-12 ^{0} C (0.5-15o C)
- Spawning March-August (May-June)
- Murawski and Finn 1988

Yellowtail Growth by Region



Yellowtail Growth by Sex



Yellowtail Maturation-Males **Yellowtail Maturation-Females**

Stock	*L50* (Males)	*L50* (Females)
SNE	19.6 cm	25.5 cm
GB	21.4 cm	25.8 cm
CC	26.8 cm	29.7 cm

<u>Age Validation Studies</u>

➢ Scales only

➢ Royce, Buller, and Premetz 1959
1. first annulus
2. 1941 and 1945 year-classes
3. marginal edge analysis

➢ Lux and Nichy 1969
1. tag-recapture
2. length frequency modal analysis

<u>Sampling Protocols</u>

❖ Scales collected from surveys, commercial landings, and sea-sampling
❖ Survey sampling protocol: 1 fish per cm per station (unsexed catch)
❖ Commercial protocol: 15 males and 15 females per 100 lengths (sexed)
❖ Sea-sampling protocol: 1 fish per cm per haul from discarded and kept catch (sexed)

Sampling and Storage

- Numerous scales from caudal peduncle area
- Stored within paper liners in coin envelopes
- Otoliths (both) stored dry in coin envelopes
- Length (cm), weight (g), sex, macroscopic maturity stage, and stomach contents and volume for selected fish during surveys
- Length and sex for commercial samples

Preparation and Equipment

- Scale impressions using laminated plastic and jeweler's press
- Otoliths embedded in wax and thin-sectioned using Isomet low-speed saw
- Scales aged using micro-projectors (20-50x)
- Sections aged using microscopes (12-25x)
- Image analysis system (Olympus/Optimas 6.5), not used during production aging

Age Reading Methodology

- ♦ Length and sex information available
- ♦ Scale annulus consists of a completed zone of compressed circuli
- ♦ Otolith annulus consists of a completed hyaline zone completely bounded by opaque material
- ♦ Edge determination
- ♦ Seasonal algorithms
- ♦ Spawning and settling checks, split zones

Quality Control

1. Two readings by primary reader
2. Partial (10-50%) reading by second reader
3. Primary and secondary readers conduct percent agreement and age bias exercises
4. Comparison of mean lengths-at-age, ranges of size at age, CVs, tracking year-classes
5. Reference collection under development
6. All material archived

Website: http://www.nefsc.nmfs.gov/fbi/age-man/yt/yt.htm

References

Lux, F.E. and F.E. Nichy. 1969. Growth of yellowtail flounder, *Limanda ferruginea* Storer) on three New England fishing grounds. ICNAF Res. Bull. 6: 5-25.

Murawski, S.A. and J.T. Finn 1988. Biological basis for mixed-species fisheries: species co-distribution in relation to environmental and biotic variables. Can. J. Fish. Aquat. Sci. 45:1720-1735.

Royce, W.F., R.F. Buller, and E.D. Premetz. 1959. Decline of the yellowtail flounder (*Limanda ferruginea*) off New England. Fish. Bull. 146: 1-267.

Appendix III

Inventory of existing historical yellowtail flounder age/growth databases in the various regions.

Area	Stock Unit	Length data (commercial)	Length data (research)	Age data commercial	Age data (research)	Structures
Grand Bank (NAFC)	3LNO	1965-present	1949-present (Spring.) 1985- present (Autumn.)	1965-present	1949-present	Otoliths scales (1999-2000)
St. Pierre Bank (NAFC)	3Ps	-	1972-present	-	1972-present	otoliths
S. Gulf of St. Lawrence (GFC)	4T	1985-88, 1992, 1995-2000	1971-present	-	1972-1982	otoliths
Scotian Shelf (BIO)	4Vs, 4W 4X	1948-78, 1980-97 1948-83 1980-2000	1970-present	1948-83 1948-83 1980-81	1970-present	otoliths
Cape Cod (MDMF)			1978-present		1978-present	scales
US Mid-Atlantic, S. New England, Georges Bank, Cape Cod (NEFSC)	5&6	1957-present	1963-present (Autumn.) 1968-present (Spring.) 1992-present (Winter.)	1965-present	1963-present	scales
Georges Bank - Canada (SABS)	5Zhjm	1994-present	1987-present	1995-present	1987-1991, 1996-present	Otoliths scales (2000)

Appendix IV - Age Reading Comparisons At Various Institutes
Age Reading Comparisons at NAFC

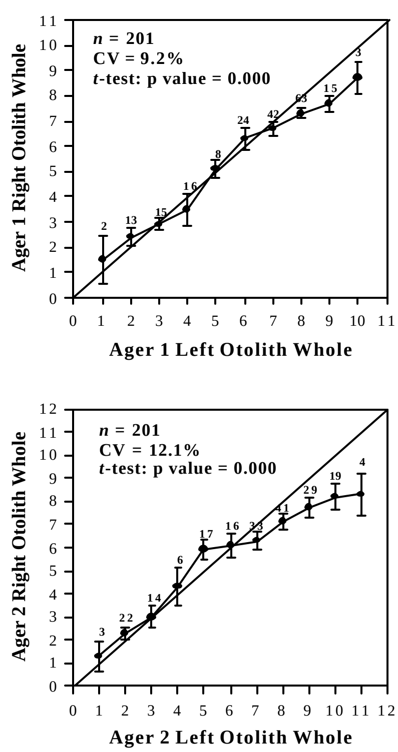
by

Karen Dwyer, Mick Veitch and Steve Walsh
Northwest Atlantic Fisheries Center, St. John's

Age reading comparisons were carried out at NAFC for the past year. Comparisons included between reader, structure, method and left/right otolith. Precision was tested using an average coefficient of variation (CV) and bias tested with age bias plots and paired *t*-tests.

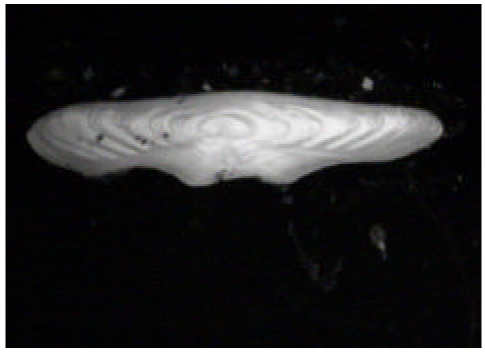
Traditionally, at NAFC, yellowtail flounder are aged using both whole otoliths and are sometimes ground, depending on the difficulty of read. The maximum age reached using this method is about 10 years. There was no bias between readers for both left and right otoliths, and CV was 8.3 and 11%, respectively. The right otolith tended to be underaged at older ages (bias at older ages) for both readers.

The effects of grinding the otolith were also tested. Reader 2 tended to overage both the left and right ground otolith in comparison with Reader 1. Grinding seemed to remove the effects of the right otolith bias, however, and



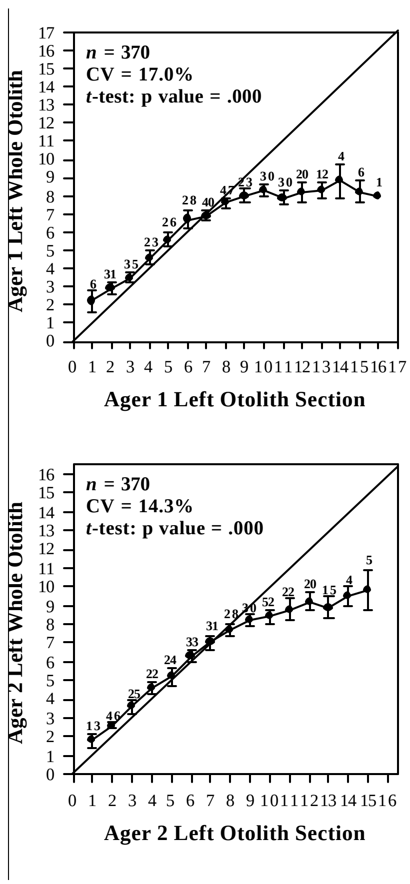
there were no differences between ages estimated by left and right otoliths. There were no differences seen for ground versus unground otoliths for either left or right otoliths, indicating that there seems to be no need of grinding at the NAFC, as it doesn't increase clarity.

Thin sectioning is assumed to be a good method for examining potential older fishes, and is done at NAFC using an Isomet low speed saw, with double diamond tipped blades to make a dorso-ventral cut. Sections are less than 0.5 mm thick. The maximum age reached using this method was 16 years old.



There was no bias seen between readers for the left otolith, but there was bias at older ages for the right otolith, with Reader 2 seeing more annuli than Reader 1. This bias was less than a year, and considered negligible, however. The CV between readers was higher for sectioned otolith readings than whole otolith readings, which is probably due to the fact that both readers are relatively inexperienced reading otoliths in this manner. Reader 1 tended to underage the right otolith sections at older ages, but Reader 2 did not. This was not unexpected, because Reader 2 has more experience ageing otoliths by this method.

A comparison between thin sections and otoliths revealed extreme bias between the two methods. Generally, after age 7, ages derived from whole otoliths tend to be much lower than those estimated from sections. There also tended to be a bias towards underageing sections at younger ages but this is thought to be due to a mis-interpretation of the first annulus.



Another method examined was baked otolith sections. The sections were placed in a muffle furnace at 400°C for approximately 2 minutes. This method is thought to enhance the annuli by making them a caramel color. There were no differences between readers and there was a CV of 8.2%. There was a significant difference between baked and unbaked sections for Reader 1 but not Reader 2; however, the bias for Reader 1 was not obvious and deemed negligible. Therefore it was concluded that there was no advantage in baking the thin sections for age estimation yellowtail flounder.

The other age estimation structure examined was scales. Acetate impressions of the scales were made and examined with a microfiche reader. Readers found scales difficult to read, as neither reader had much experience with them. There tended to be some bias at older ages, with Reader 2 underageing the older fish. Both readers showed a strong bias after age 8 (departure from the 1:1 equivalency line) when comparing whole otoliths and scales, with whole otoliths giving a younger age than scales. Again, there seemed to be a mis-interpretation of the first annulus in scales. After age 7, there was also a difference between scales and thin sections, with both readers tending to see fewer annuli in scales than sections.

Without age validation, it is difficult to say which structure or method gives the most accurate age. However, it is likely that annuli read from the internal surface of the otolith are closest, and have been validated by many other authors (English sole (*Parophrys vetulus*) (McLellan and Fargo, 1995); redfish (*Sebastes mentella*) (Campana *et al*., 1990); haddock (*Melanogrammus aeglefinus*) (Campana, 1997)). However, it seems that either scales or whole otoliths may be used and preparation is less labour-intensive than thin sections for fish up to age 7. In order to decrease the CV between readers, especially for thin sections it is recommended that readers draft a set of protocols for reading thin sections, and the first annulus be validated to decrease bias in younger fish.

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MacLellan, S. E. and J. Fargo. 1995. Validation of age and growth for English sole (*Parophrys vetulus*) in Hecate Strait, British Columbia. In: Recent Developments in Fish Otolith Research. D. H. Secor, J. M. Dean, S. E. Campana (Eds.). University of South Carolina Press: SC, USA.

The use of acetate replication and calcified structure age and growth extraction software (CSAGES) in the age interpretation of yellowtail flounder otoliths

by

Robert Perry
Inland Fish and Wildlife Division, Department of Forest Resources and Agra foods
Government of Newfoundland and Labrador

The Provincial Government of Newfoundland is currently using techniques and standards that may assist in the aging of older yellowtail flounder.

Cellulose acetate replication of sagittal otoliths

Dr John Casselman, Senior Research Scientist, with the Ontario Ministry of Natural Resources developed the technique of acetate replication. The technique involves the creation of a transparent replication of the sagittal otolith surface. The advantage in creating a transparency is that it eliminates opacity, normally associated with direct interpretation from the otolith surface; thereby ensuring that topographic detail, previously unseen, is revealed.

Specifically, the technique is good for age estimation of older fish. With older fish the outer annuli tend to coalesce near the outer margin of the structure, making it difficult to differentiate between annual increments. The increased transparency gives the interpreter the ability to differentiate between annual year marks. The increased transparency also allows for the use of higher magnification.

Brief overview for the preparation of acetate replicates

Embedding

Otoliths are embedded in a resin/epoxy mixture that, upon drying, is very hard. Embedding the otolith increases the ease of handling and sectioning. Chipping and cracking normally associated with thin sectioning is also reduced.

Sectioning and polishing

A thin section (400-450? m) of the embedded otolith is cut, transversely, using an isomet low speed saw equipped with diamond tipped wafer blades. To ensure the proper thickness, measured plastic spacers are used to separate the two wafer blades. The section is oriented through the origin at right angles to the maximum long axis of the otolith. The embedded section is mounted on a glass slide and polished smooth using successively finer grades of sandpaper.

Etching and replication

The smooth surface of the mounted section is then etched using a mild acid. Following etching the surface of the otolith is replicated using acetone. The acetone is placed on the surface of the otolith. A small piece of cellulose acetate is quickly rolled onto the surface of the section. When the acetate is removed from the surface of the section a replicate of the otolith surface will have been impressed in the acetate. The replicate is then placed between two glass slides in preparation for interpretation.

Age interpretation and database

The Newfoundland provincial government has adopted an age interpretation and archival program from the Ontario Ministry of Natural Resources. Individual annual increments are measured and enumerated with a Calcified Structure Age and Growth Extraction software (**CSAGES)** (Casselman and Scott 2000). The program has several utilities including an archival component and a function that allows for the comparison of individual interpretations.

Using transmitted light each otolith replicate is examined under a microscope equipped with drawing tube (100x magnification). The replicate image is reflected through the drawing tube and projected on a digitizing tablet. Secured to the digitizing tablet is a radial line. The reflected image is aligned so that the axis of interpretation overlies the radial line. Individual annuli are measured and recorded by CSAGES where they intersect with the radial line.

The advantages in using this program are as follows:

(1) The program provides a set standard of protocols and definitions to interpret individual growth increments. Thus, it ensures that there is consistency for terminology associated with age interpretation and that data recorded by each reader is done in a standardized manner.

(e.g.)Age reading terminology adopted by the Newfoundland Provincial Government to describe annuli and observed incremental growth(Casselman and Scott 2000)

Alpha : Indicates that the check or translucent zone associated with the annulus is demarcated by a small amount of tissue or mineral (new growth). Is present in the most distal parts of the structure but not all regions (annulus just forming).

Omega: denotes that the check or translucent zone that is associated with the annulus is present on the edge but is not demarcated by tissue or mineral, hence the annulus is completely formed.

Plus growth condition (+): moderate number of circuli on the edge outside the last annulus or relatively wide zone of opaque tissue or mineral along or around the entire edge of the structure. The zone is relatively narrow, < 50% of the width of the proceeding annual zone or increment.

Plus growth condition (++): large number of circuli on the edge outside the last annulus or relatively wide zone of opaque tissue or mineral along or around the entire edge of the structure. The zone is relatively wide, >50% of the width of the preceding annual zone or increment

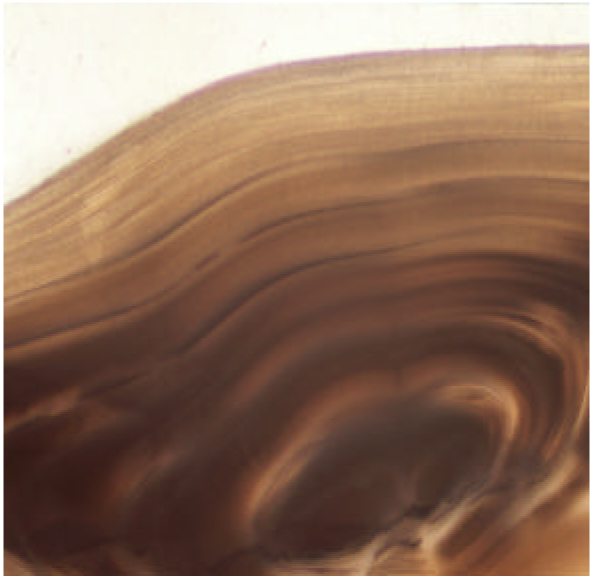
(2) By using the program all individual annuli measurements and age interpretations are recorded and archived in a central location. Data can also be extracted in spreadsheet form. This is beneficial when investigating age and growth or precision and bias. Further, it also ensures all samples, when needed, can be used for reference and teaching purposes

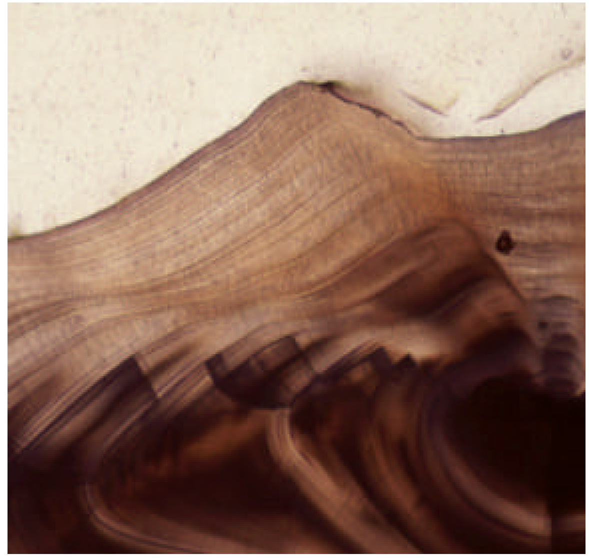
For more information on the acetate replicate technique and CSAGES contact:

Dr John Casselman
Senior Research Scientist
Science Development and Tranfer Branch
Aquatic Ecosystems Science Section
Glenora Fisheries Station
R.R. 4, Picton Ontario
K0K 2T0

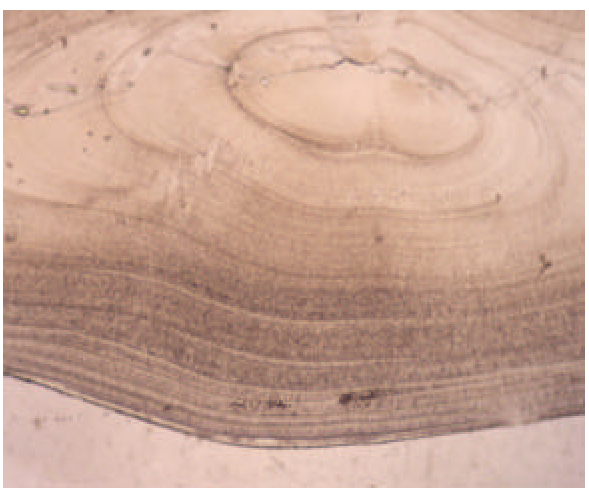
References

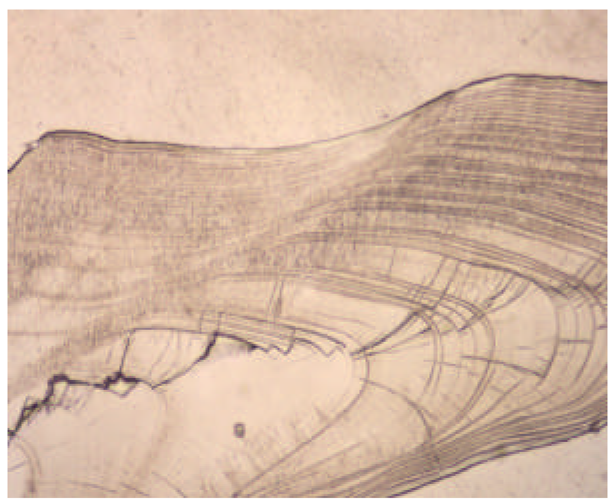
Casselman, J.M., and K.A. Scott. 2000. A General Procedures Manual for CSAGES—Calcified Structure Age-Growth data Extraction Software(Version 5.2). Special Publication of the Glenora Fishereis Station, Ontario Ministry of Natural Resources, Picton Ontario.90p.





The age estimation from these acetate replicate images (above) of yellowtail flounder when compared to those ages derived from the thin sections (below) of the same otolith in some cases gave higher age readings. Further comparative work is expected.





Georges Bank Yellowtail Flounder:

Comparison of Age Readings from Whole Otoliths, Scales and Otolith Cross-Sections

by

Heath Stone and Peter Perley
St. Andrews Biological Station, St. Andrews

- Based on a sample collected from the February 1998 Georges Bank Groundfish Survey **(*n*=55)**, and a commercial port sample obtained in October, 2000 **(*n*=27)**.
- For the survey sample, we compared age readings from **whole otoliths** and **scales** only. (Note: Our 1st attempt at cross-sectioning these otoliths was not successful).
- For the commercial sample, we compared age readings from **whole otoliths, scales** and **otolith cross-sections**.

Comparison of Age Determinations: RV Survey Sample, February 1998 (*n*=55)

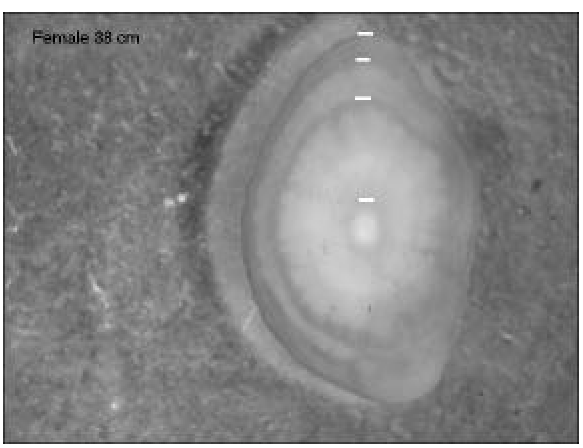
- Percent agreement between otoliths and scales was 71%.
- Ages determined from whole otoliths had a tendency to be lower than those from scales after age 3.

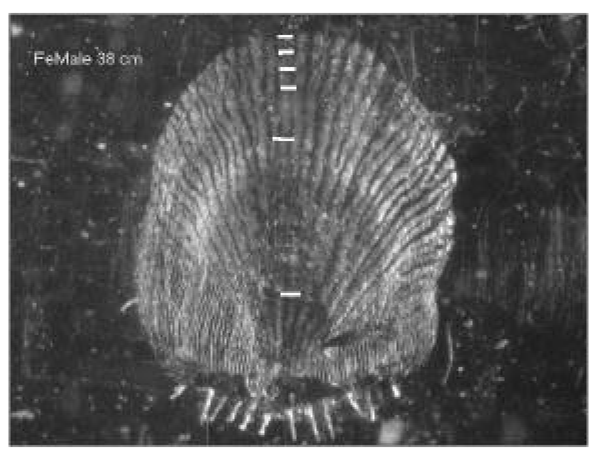
Comparison of Whole Otolith Age vs Scale Age

Whole Otolith Age	Scale Age 2	3	4	5	6	Total
2	5	1				6
3		17	5			22
4		1	12	8	1	22
5				4		4
6					1	1
Total	5	19	17	12	2	55

(71% Agreement)

Otolith age 4 vs Scale age 6: Female 38 cm





Comparison of Age Determinations: Commercial Sample, October 2000 (*n*=27)

- Percent agreement for **scales vs otolith cross-sections was 67%** and showed less bias than the other comparisons.
- Whole otliths tended to be **underaged** relative to the scales and cross-sections, particularly at ages 4 and older

Comparison of Whole Otolith Age vs Scale Age

Whole Otolith Age	Scale Age 2	3	4	5	6	7	Total
2	8		1				9
3		3	1		1		5
4			3	3			6
5				1	5		6
6						1	1
Total	8	3	5	4	6	1	27

(56% Agreement)

Comparison of Whole Otolith Age vs Otolith Cross-Section Age

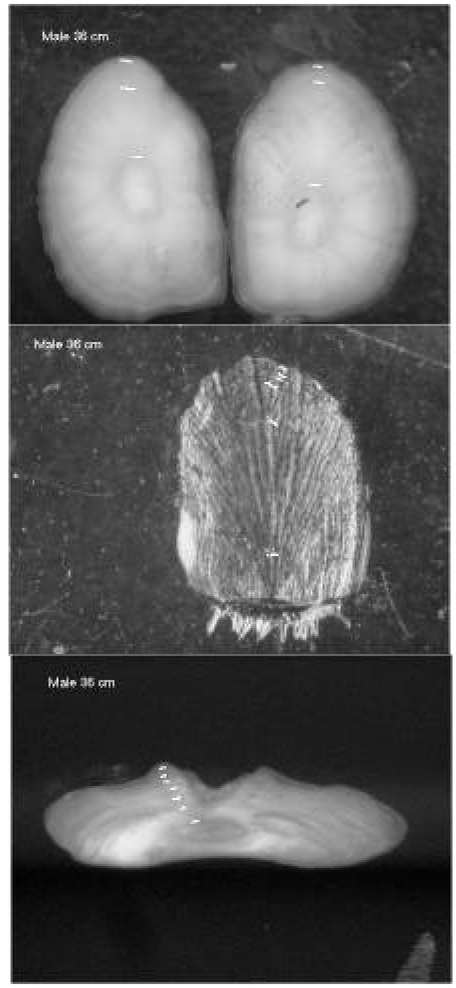
Whole Otolith Age	Otolith Cross-Section Age 2	3	4	5	6	7	Total
2	8	1					9
3		3	1			1	5
4		1		4	1		6
5				3	3		6
6						1	1
Total	8	5	1	7	4	2	27

(52% Agreement)

Comparison of Otolith Cross-Section Age vs Scale Age

Otolith X-S Age	Scale Age 2	3	4	5	6	7	Total
2	7		1				8
3	1	3	1				5
4			1				1
5			2	3	2		7
6				1	3		4
7					1	1	2
Total	8	3	5	4	6	1	27

(67% Agreement)



Otolith age 3 vs Scale age 6 vs X-Section age 7: Male 36 cm

Conclusions and Recommendations:

- Although sample sizes were very small for these comparisons, scales and otolith cross-sections may show more annuli than whole otoliths, especially after age 3.

- Need to explore further the preparation techniques and interpretation of otolith cross sections.

- Need to determine best area for age reading on otolith cross-sections (i.e. along the edge of the sulcus or laterally along the section).

- Require some assistance in determining ages from scales to make sure our interpretations are correct

Apppendix V - Accuracy, Precision and Age Validation
Accuracy, Precision and Age Validation at NAFC

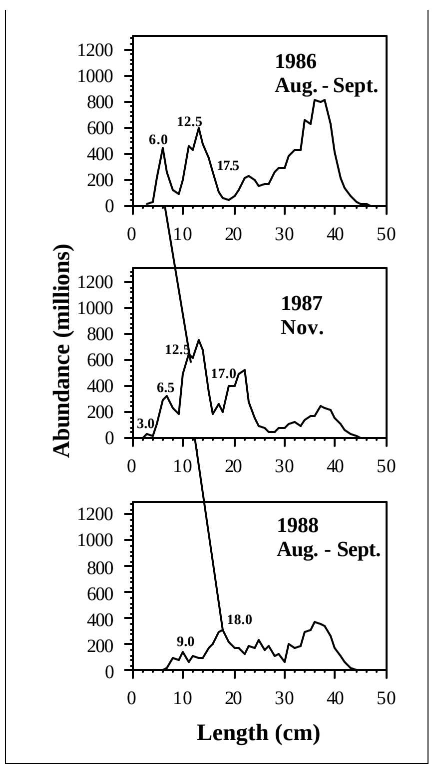
by

Karen Dwyer, Steve Walsh and Mick Veitch
Northwest Atlantic Fisheries Centre, St. John's

A number of indirect methods of age validation have been examined over the last year at NAFC. There are plans for some direct age validation methods in the future but to date, there has only been some tagging work done. Tagging has revealed, however, that time at liberty corroborates ages obtained by using thin sectioned otoliths.

Interpretation of the first annulus was examined by collecting young of year fish in the spring (when the annulus is apparently laid down) and measuring the width and thickness of the otolith section in order to get the dimensions of the first annulus. These measurements were 1.1 mm x 0.5 mm and this dimension was verified in the older fish as being the first annulus. Some one-year-old otoliths revealed a strong ring inside the first annulus, which made interpretation difficult, but this may have been a settling check. It is advised that some work with microstructure of the otolith be done and the daily rings counted in order to validate the first annulus. Note: since then, the first annulus has been found to be much smaller than originally thought, and is about 0.65 mm

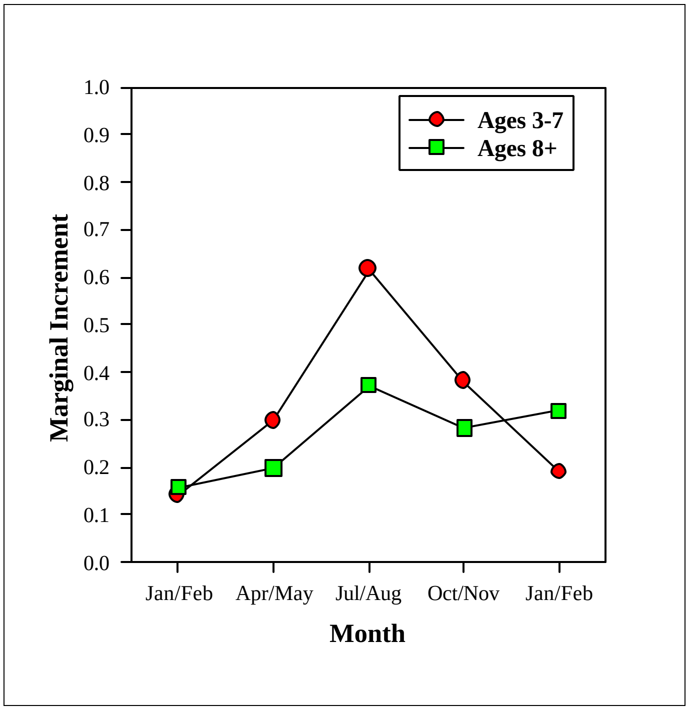
Length frequency analysis was useful in validating young fish. Ichthyoplankton surveys (Frank *et al*., 1992) revealed a mode in September, which corresponded to pelagic 0-group fish. These fish were all under 3.5 cm. Regular annual surveys in 1985-1994 were used to determine modal lengths of fish from the one, two and sometimes three age classes. In particular, strong year classes such as that in 1985 could be followed in the years 1986-1988. Modes from these years revealed that demersal 0-group yellowtail were about 3-4 cm, indicating metamorphosis at about 3-3.5 cm. The one-year-old mode was strong, and the size of fish of this age was estimated to be approximately 7 cm in males and females. The size of age 2 year old fish was estimated be 13 cm and 14 cm in males and females, respectively.



A comparison of length frequency values and length at age values derived from different methods show that readers tend to assign older fish to younger year classes (1-5) for both sections and scales, while whole otoliths tend to be similar to the length frequency values. Again this indicates a need to determine the first annulus.

Marginal increment analysis for sections showed that younger fish (3-7 year olds) show a slight pattern in the marginal increment of the otolith over a year's cycle. This may mean that material is deposited to the edge of the otolith in an annular fashion. However, this pattern is not strong, and is even less strong for older fish.

Obviously it is difficult to compare otolith growth between wild and cultured yellowtail flounder; however it seems that both whole and sectioned otoliths from cultured yellowtail verify that under optimum growing conditions, yellowtail lay down one annulus per year. It also seems that the annulus is laid down sometime before April.



Quality control at NAFC is, for the most part, lacking. There is one age reader per species and the only reference collection available is for yellowtail flounder. It is still under development. Training protocols for new agers tend to follow guidelines that have been set at other institutions. To measure bias between readers, age bias plots and *t*-tests should be used, and to measure precision, CV should be used. At the moment, only percent agreement is used in cod age studies

We are reasonably confident in our ability to age whole otoliths correctly up to about age 7, and we have demonstrated indirect validation of all three methods (scales, sections and whole otoliths) up to this age. At NAFC, quality control must be emphasized and encouraged, and some direct forms of age validation carried out to ensure thin sections are accurate for older ages. Finally a reference collection should be picked and used regularly.

References

Frank, K.T., J.E. Carscadden, W.C. Leggett, and C.T. Taggart. 1992. Larval flatfish distributions and drift on the southern Grand Bank. *Can. J. Fish. Aquat. Sci*. 49: 467-483.

Appendix VI - Annotated Images From Each Region

Grand Bank Otoliths – fish captured in fall, 1999.

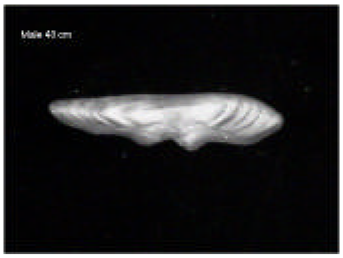


Figure 1A. Age estimated by panel for section 8 years.

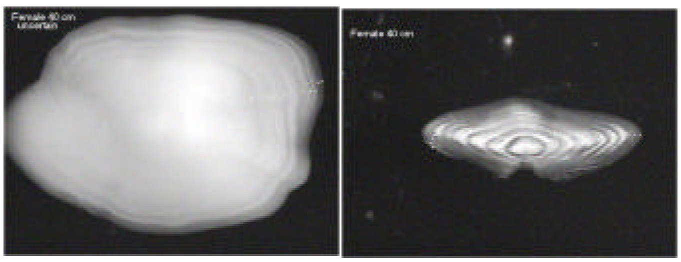


Figure 1B. Age estimated by panel for whole otolith uncertain; for section 9 years.

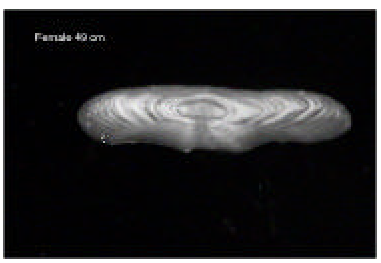


Figure 1C. Age estimated by panel for section 12 years.

Figure 1D. Age estimated by panel for section 2 years.

Figure 1E. Age estimated by panel for section 4 years.

<u>Southern Gulf Otoliths – fish captured in September 2000</u>

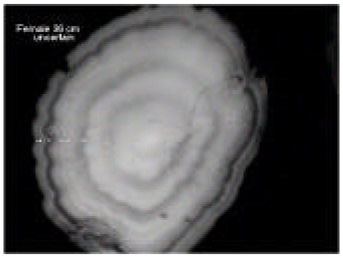


Figure 2A. Age estimated by panel for whole otolith uncertain (age 7?).

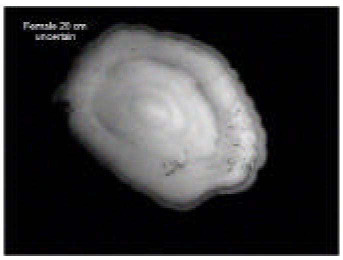


Figure 2B. Age estimated by panel for whole otolith uncertain (age 3?).

Figure 2C. Age estimated by panel for whole otolith 3 years.

Scotian Shelf Otoliths – fish captured in July, 1979.

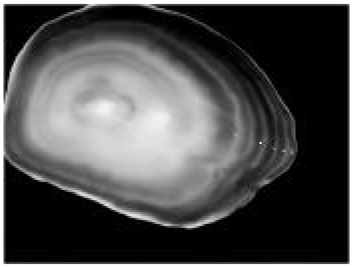


Figure 3A. Age estimated by panel for whole otolith 7 years.*

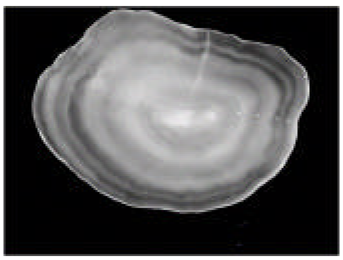


Figure 3B. Age estimated by panel for whole otolith 5 years.

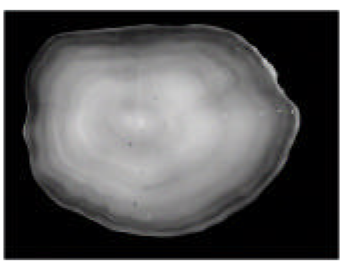


Figure 3C. Age estimated by panel for whole otolith 5 years.

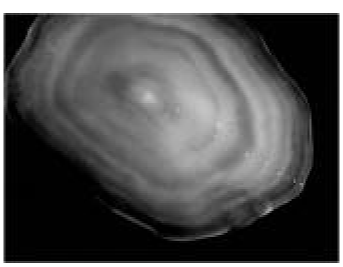


Figure 3D. Age estimated by panel for whole otolith 8 years.

* Length and sex of fish unknown.

<u>Canadian Georges Bank Otoliths/Scales – fish captured in October, 2000</u>

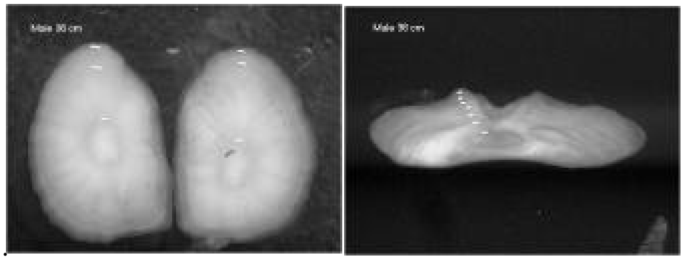


Figure 4A. Age estimated by panel for whole otolith 3 years; for section 7 years.

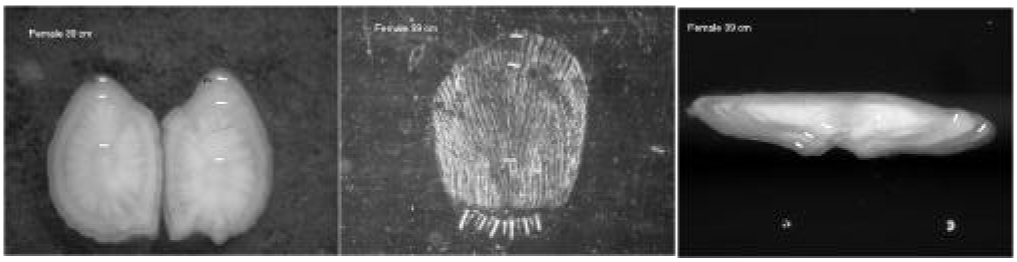


Figure 4B. Age estimated by panel for whole otolith 3 years; for scale 3 years and for section 3 years.

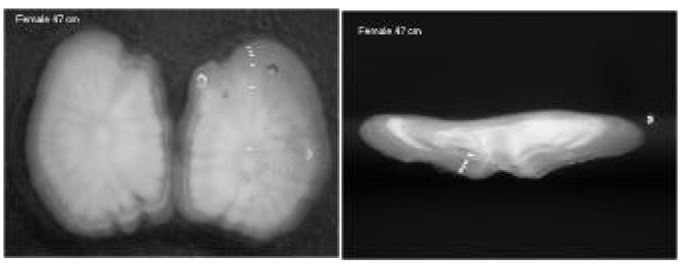


Figure 4C. Age estimated by panel for whole otolith 6 years; for section 7 years.

Figure 4D. Age estimated by panel for whole otolith 4 years; for section 7 years.

<u>United States Georges Bank Otoliths – fish captured in fall, 2000</u>

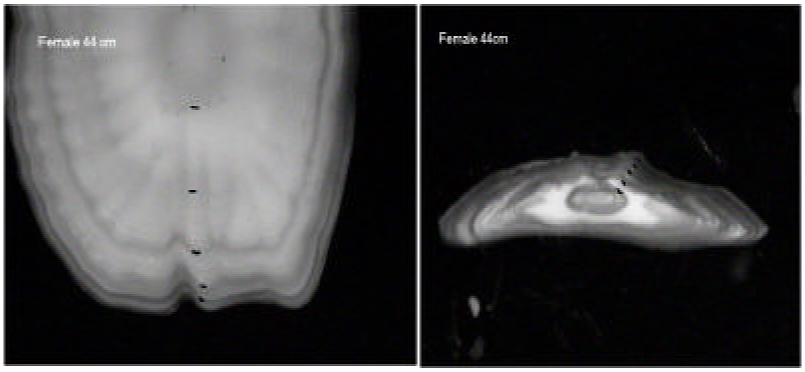


Figure 5A. Age estimated by panel for whole otolith 5 years; for section 5 years.

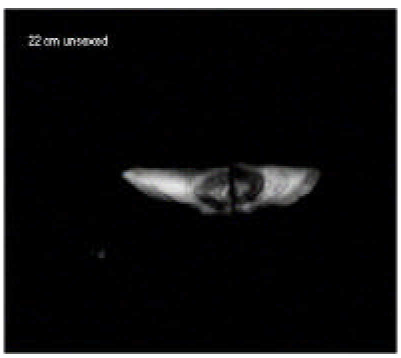


Figure 5B. Age estimated by panel for section 1 year.

Appendix VII. Workshop Participants

Front row (l-r): **Mark Fowler, Vaughn Silva, Heath Stone, Karen Dwyer, and Isabelle Forest.** ***Back row (l-r):*** **Steve Campana, Steve Walsh, Jeremy King, Jay Burnett, Rob Perry, Peter Perley and Mike Veitch,** ***St. John's harbour in the background.***

Karen Dwyer, (Organizer)
Northwest Atlantic Fisheries Centre,
Dept. Fisheries and Oceans,
Science Oceans and Environment Branch
Aquatic Resources Division,
Flatfish and Deepwater Species Section
P.O. Box 5667,
St. John's, NF A1C 5X1
Karen Dwyer [karen_dwyer@hotmail.com]

Jay Burnett, (Convener)
Northeast Fisheries Science Center,
National Marine Fisheries Service,
166 Water Street, Woods Hole,
Massachusetts, 02543 United States.
Jay Burnett [jburnett@whsun1.wh.whoi.edu]

Steve Campana, (Convener)
Marine Fish Division
Bedford Institute of Oceanography,
P.O. Box 1006
Dartmouth, N.S. Canada B2Y 4A2.
campanas@mar.dfo-mpo.gc.ca

Isabelle Forest,
Gulf Fisheries Centre,
Dept. Fisheries and Oceans
343 Ave Université,
Moncton N.B Canada E1C 9B6.
Foresti@dfo-mpo.gc.ca

Mark Fowler,
Marine Fish Division
Bedford Institute of Oceanography,
P.O. Box 1006
Dartmouth, N.S.
Canada B2Y 4A2.
fowlerm@mar.dfo-mpo.gc.ca

Jeremy King,
Massachusetts Division of Marine Fisheries,
50A Portside Dr.,
Pocasset, MA 02559
Jeremy King <jeremy.king@state.ma.us>

Peter Perley,
St. Andrews Biological Station
Dept. Fisheries and Oceans
531 Brandy Cove Road,
St. Andrews, N.B
Canada E5B 2L9.
perleyp@dfo-mpo.gc.ca

Robert Perry,
Inland Fish and Wildlife Division
Department of Forest Resources and Agrafoods
Government of Newfoundland and Labrador
Rob Perry [RobPerry@mail.gov.nf.ca]

Vaughn Silva,
Northeast Fisheries Science Center,
National Marine Fisheries Service,
166 Water Street, Woods Hole,
Massachusetts, 02543 United States.
vsilva@whsun1.wh.whoi.edu

Heath Stone,
St. Andrews Biological Station
Dept. Fisheries and Oceans
531 Brandy Cove Road,
St. Andrews, N.B
Canada E5B 2L9.
stoneh@dfo-mpo.gc.ca

Mick Veitch,
Northwest Atlantic Fisheries Centre,
Dept. Fisheries and Oceans,
Aquatic Resources Division,
Flatfish and Deepwater Species Section.
P.O. Box 5667, St. John's,
NF A1C 5X1
veitchm@dfo-mpo.gc.ca

Stephen J. Walsh, (Convener
Northwest Atlantic Fisheries Centre,
Dept. Fisheries and Oceans,
Aquatic Resources Division,
Flatfish and Deepwater Species Section.
P.O. Box 5667, St. John's,
NF A1C 5X1
walshs@dfo-mpo.gc.ca