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The Canada-United States Yellowtail Flounder Age Reading Workshop

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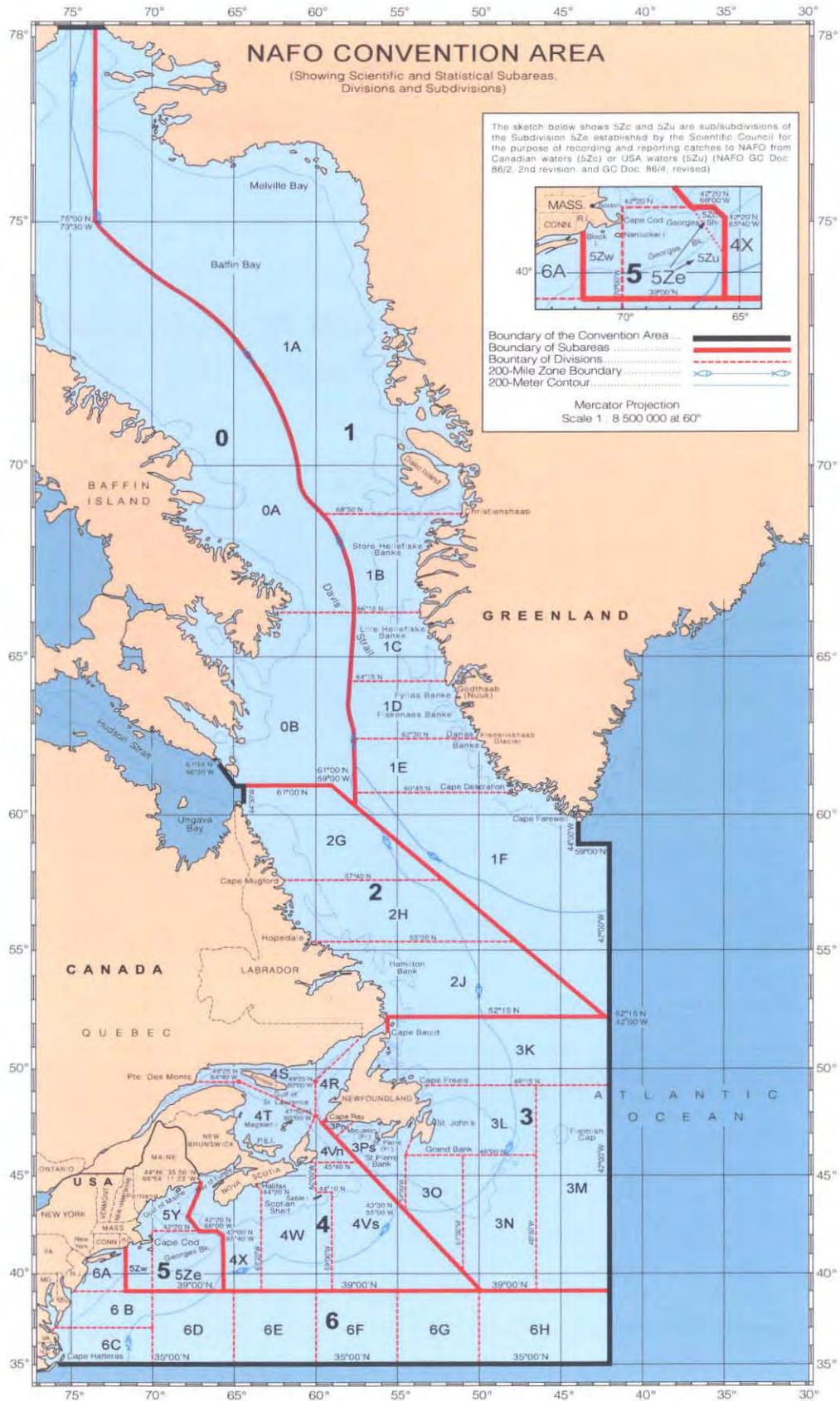
Foreword

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

This issue of *NAFO Scientific Council Studies* contains the proceedings and papers presented at *The Canada-United States Yellowtail Flounder Age Reading Workshop* as proposed for publication by the Standing Committee on Publications (STACPUB) of the Scientific Council. The 34 previous issues of the *NAFO Scientific Council Studies* are listed on the back cover of this volume.

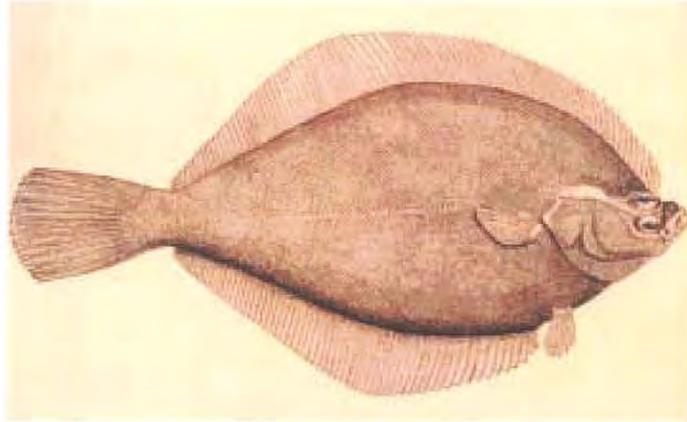
December, 2002

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

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Abstract

This report records the presentations and discussions from the Canada-United States Yellowtail Flounder Age Reading Workshop held in St. John's, Newfoundland, 28-30 November 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 (scales and otoliths) used in ageing yellowtail flounder (*Limanda ferruginea*) 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 on developing a minimum set of standards for ageing, training, reference collection organization, statistical analysis and quality control. It concluded that slow growing coldwater species are often under-aged using the whole otolith method. Progress on implementation of these recommendations will be reviewed in 3 years.

Key words: Ageing, yellowtail flounder, otoliths, scales

Introduction

For many years, the accuracy of determining the ages of older yellowtail flounder (*Limanda ferruginea*) at the Northwest Atlantic Fisheries Center (NAFC), Newfoundland Region of the Department of Fisheries and Oceans (DFO) Canada, has been in doubt. Several tag returns (including some otoliths) from an 1990-93 Petersen disc tagging experiment on the Grand Bank confirmed that yellowtail flounder were living longer than indicated from the current age readings (Walsh and Morgan, MS 1999). In 1999, communications were established with researchers involved in ageing yellowtail flounder at DFO institutes in eastern Canada and also in the Massachusetts area of the eastern United States with the purpose of holding an age reading Workshop. 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 have recently begun ageing 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, are interested in starting ageing 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). The following were the Terms of Reference:

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

Twelve age readers attended the Workshop (see List of Participants at Appendix II). The age reading protocols of NAFC, Massachusetts Division of Marine Fisheries (MDMF), NEFSC, SABS, GFC, Inland Fish and Wildlife Division (IFWD) and BIO were compared and discussed. A common set of minimum standards and protocols for ageing, training, reference collection organization, statistical analysis and quality control were developed and recommendations for future work were considered.

The Workshop

The Workshop was held in St. John's, Newfoundland, 28–30 November 2000 and 12 age readers from NAFC, MDMF NEFSC, SABS, GFC, IFWD and BIO attended. In preparation for the Workshop, age readers at NAFC, SABS and NEFSC conducted comparison of ages from whole and sectioned otoliths with scales from yellowtail flounder stocks in their respective regions. During the course of the Workshop, participants also conducted blind readings of various otolith and scale samples from all regions. Several recommendations were made regarding age validation, quality control and reference collections.

The format of the Workshop report is structured to reflect the presentation of the main points of the results, discussion, proposals and recommendations. A full version of each presentation is appended to this Workshop report (see Appendix III).

Importance of Age Estimation

The Workshop began with a general discussion on the importance of accurate age readings and age validation. The age estimation of many marine fishes is normally obtained by reading scales and otoliths for annual increments. Yellowtail flounder are primarily aged by scale readings in the eastern United States and by otolith readings in eastern Canada. Age validation is required to determine whether these increments are actually annual. Some of the analytical models used in the assessment of yellowtail flounder are age based and rely on accurate age estimation of fish samples. Errors in age estimation 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.

General Biology

Each institute presented an introductory overview of the biology and ecology of yellowtail flounder stocks in their area and the current age related research programs being carried out in their respective areas (see Appendix III for summaries).

Distribution. Yellowtail flounder is a small-mouthed right-eyed flounder distributed in the Northwest Atlantic from southern Labrador to Chesapeake Bay (Fig. 1). The most northerly distribution of commercial quantities is found on the Grand Bank, NAFO Div. 3LNO, off the coast of Newfoundland. There is a small, unregulated stock on St. Pierre Bank NAFO Div. 3Ps 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, MS 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 bottoms at depths of 20–70 m (10–40 fathoms) and are capable of tolerating a wide range of temperatures, -1.2° to 15°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.

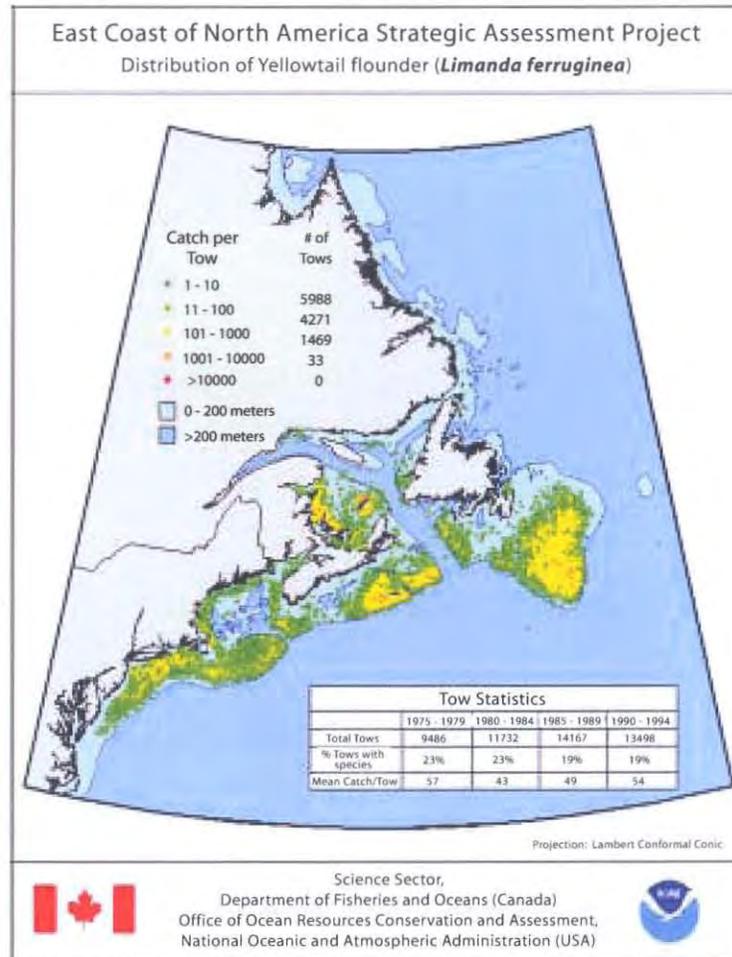


Fig. 1. Distribution and relative abundance of yellowtail flounder in the North-west Atlantic.

Age, growth and maturity. As with most flounders, yellowtail flounder growth rates are sexually dimorphic, with males growing slower beginning with the onset of sexual maturity and reaching a smaller maximum size, than females. Across the entire range of distribution, growth varies latitudinally, with faster growing fish, but reaching a smaller maximum, occurring in the southern regions off the eastern USA and slower growing and reaching a larger maximum size characteristic of fish in northern regions, i.e. the Grand Bank stock. Within regions, differences in growth are also evident. The largest 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 Bank, historically, yellowtail flounder have been aged up to age 14 years, although there is now growing evidence that suggests they may reach much older ages. Although a maximum age of 17 years has been reported, few fish in U.S. waters exceed age 8 or 9 years. Yellowtail flounder from the southern Gulf of St. Lawrence and the Scotian Shelf are presently not aged.

On the Grand Bank, males mature at a median age (A_{50}) of 4 years and a median length (L_{50}) of 25 cm and females mature at a 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 $A_{50} = 1.8$ years and $L_{50} = 19.6$ cm, and

females mature at $A_{50} = 1.6$ years, $L_{50} = 25.5$ cm. Georges Bank males mature at $A_{50} = 1.3$ years, $L_{50} = 21.4$ cm and females mature at $A_{50} = 1.8$ years, $L_{50} = 25.8$ cm. Cape Cod males mature at $A_{50} = 2.6$ years, $L_{50} = 26.8$ cm and females also at $A_{50} = 2.6$ years, $L_{50} = 29.7$ cm (O'Brien *et al.*, 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.

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, difficult to read otoliths are ground with a flat abrasive stone. Both sex and length information is available. There is no distinction made on which otolith to read (i.e. left vs right). The edge is read and coded but it is not used and neither is the birthday of January 1 used. Current sampling protocol 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 and 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 protocol established for American plaice (*Hippoglossoides platessoides*) be applied to yellowtail flounder. For American plaice, whole otoliths are read. A reference collection is used by the primary age reader to calibrate their reading before beginning a survey collection. Knowledge of the month of capture is available but length and sex of the fish are not. The edge is defined and the size calculated.

BIO: Yellowtail flounder otoliths were collected during research surveys since 1970. Only otoliths collected through 1970–83 have been read. Otoliths are stored in a glycerine/thymol solution. No maturity data has been collected since 1985. This species is assessed together with American plaice and witch flounder as a flatfish 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 bacterial growth. This activity will be discontinued since long-term storage in glycerine/thymol is suspected to 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 estimation.

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 estimation 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 NEFSC has aged yellowtail flounder 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: the 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 exercises. 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 of 1985-99 indicate that the commercial catch is mostly (93%) composed of ages 2-4 (Cadrin, MS 2000).

General discussion

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

TABLE 1. Various techniques currently use by the different institutes.

Institution	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 manpower resources have deteriorated the quality control in age reading in Canadian institutes. The inventory of age estimation material for each institute is listed in Appendix IV. Most of the otoliths collected in the Canadian areas have not been aged.

Standardization of Age Reading Terminology

The Workshop reviewed the glossary for otoliths (Secor *et al.*, 1995) and scales (Penttila, 1988) and agreed to use the following definitions when making reference to yellowtail flounder otoliths and scales. Specific references to scales are in italics.

Figure 2 illustrates some of the morphology of the common otolith and scale.

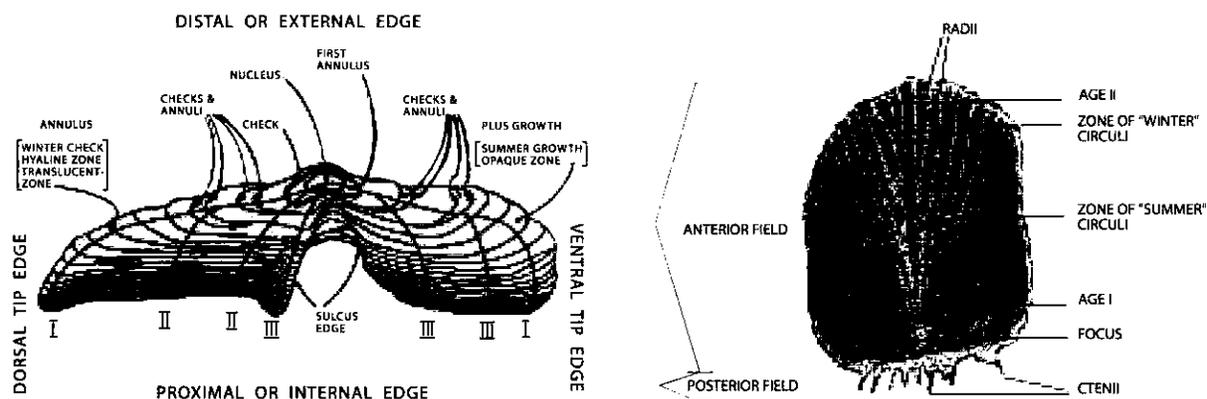


Fig. 2. Morphology of an otolith (adopted from MacLelland, 1997) and a scale (adopted from Jearld, 1983).

- 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 ageing (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 (e.g. 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 estimation 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 (e.g. subdaily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry and period of formation can vary widely. An annual increment comprises an opaque zone and a translucent zone. Annual increments can be complex structures, comprising multiple opaque and translucent zones.
- 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 estimation 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 lapillus. 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 (e.g. 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 (e.g. 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.

Standardization of Age Reading Protocols

The Workshop discussed the various sampling and storing procedures, preparation techniques and reading protocols and agreed that the following standard protocols be used when making reference to yellowtail flounder scale and otoliths and interpretation of their ages:

- 1) *Prior information on fish length* – after some debate it was felt that having this information was a matter of choice.
- 2) *Which otolith, left or right* – it was recommended that the left otolith be the standard otolith used to age yellowtail flounder since this is widely used by most participants. The right otolith could be used as a verification of the age estimation. *Which side of otolith, dorsal or ventral* – after much debate it was felt that most people used the ventral side.
- 3) *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 8–16× was required for whole otoliths and a higher magnification in the range of 25–40X was generally used for sectioned otoliths.
- 4) *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.
- 5) *Definition of nucleus* – it was critical that sectioning cut transversely through the otolith to see the nucleus. After sectioning both remaining halves should be retained for further examination if possible.
- 6) *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 year's growth was seen to differ in sizes for all stocks considered, being wider in the south and narrower in the north.

- 7) *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%.
- 8) *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 that the skin mucus be scraped off 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 estimations were complete, but after this they should be transferred to coin envelopes for long term dry storage.

Age Reading Comparisons Between Scales and Otoliths

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 V for details).

NEFSC (Jay Burnett): Yellowtail flounder in U.S waters have historically been aged using scales. As preparation for this Workshop, the Age and Growth Unit of the NEFSC 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 estimations 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 flounder age estimation, 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 flounder 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 estimations 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 estimations 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.

The Workshop noted 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. It was noted that Scotian Shelf otoliths examined at this Workshop were stored in glycerine for over 20 years. Despite this, it was agreed that otoliths could be soaked in glycerine for short periods until age estimations are complete, but thereafter, 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.

SABS (Heath Stone): Age readers at SABS conducted a comparison study of age estimations using whole otoliths, scales and otolith thin-sections for Georges Bank yellowtail flounder 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 estimations 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.

The Workshop noted that otolith preparation time could be saved if it could be established that whole otoliths could be used as the primary method up to a certain age, and thereafter thin sections would be used. It also noted that mass production sectioning of otoliths, could result in the nucleus of some otoliths being missed and therefore lead to inaccurate age estimation.

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 estimations, 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 underaging the fish when compared to thin sections from the otoliths. It is concluded that whole otoliths could be used for age estimations 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 and taking measurements of the diameter of the presumed first annulus. Scales from Grand Bank yellowtail flounder were examined by NEFSC age readers and were found to be fairly easy to read up to age 7.

The Workshop noted that the difficulty with the first annulus needs to be resolved to decrease bias in the ageing of younger fish. The *Workshop concluded* that exchanges of scales with the NEFSC lab should continue.

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 involves several steps which include 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. It was believed this method would be useful for producing good quality images, particularly for older fish, but would be too time consuming for production ageing.

The Workshop noted this is a very promising technique and more comparisons between this method and the traditional thin sections are required especially in light of the fact that ages estimated from the acetate peel are higher than those obtained from thin sections.

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 is encouraged for comparison of results within stocks and between stocks

Accuracy, Precision and Quality Control in Age Estimation

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 estimation, including a review of the use and abuse of age validation methods. The following is a synopsis:

Age validation refers to the accuracy of the ageing 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:

- 1) Release of known age fish into the wild, which can validate absolute ages.
- 2) Bomb radiocarbon dating of fish that were hatched prior to 1965.
- 3) Mark-recapture of chemically tagged fish such as those marked with oxytetracycline.
- 4) Radiochemical dating validates absolute ages and can be applied to long-lived fish.
- 5) Ageing of discrete length modes is probably best suited for younger fish.
- 6) Use of natural markers can validate absolute ages.
- 7) Marginal Increment Analysis validates growth increments but is really only suitable for fast growing fish. Older fish are always a problem.
- 8) 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.

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

- 1) 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);
- 2) Ralston method (use of daily increment widths to determine yearly age); and

- 3) Elemental isotope cycles.

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

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

Measures of precision: the best include CVs, 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.

Steps to a successful ageing program:

- 1) Choose appropriate structure(s) for age estimation;
- 2) Develop the ageing method best suited to the age structure;
- 3) Carry out age validation studies;
- 4) Prepare a reference collection and use it – an ideal reference collection derives from the age validation studies in Step III; and
- 5) Conduct a quality control program on your age reading.

Quality Control (QC) 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. The following are the components of a QC program:

- 1) *Reference collection* – ideally consists of known-age (i.e. validated) otoliths or alternatively consists of otoliths aged by internationally 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 CVs* – 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:

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

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 VI). These are summarized here.

NAFC (Karen Dwyer): Under quality control and age validation protocols for the ageing of Grand Bank yellowtail flounder the current "Indirect Methods" include 1) inspection and determination of the 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) 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 reference collections are not used. Training protocols for new age readers tend to follow guidelines that have been set at other institutions.

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.

Image analysis of otoliths and scales

The Workshop devoted several hours to review images of otoliths and scales from various stocks projected onto a screen. It was obvious that there are differences in shapes of these structures from different regions 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 VII.

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 are 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.

Discussion of Steps to a Successful Age Estimation Program

The Workshop extended its discussions on the accuracy, precision, validation and quality control in ageing. Many of the discussion topics are cross-referenced in the Section under "Standardization of age reading protocols". The discussion also included several general conclusions and recommendations.

Developing the age estimation method

- 1) *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 does not know, *a priori*, which ages are correct, but because the thin sections are the accepted method in literature for age estimation of many species 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.
- 2) *Single structure for all stocks:* For the Grand Bank stock, it appears that thin sections are the best for older fish, but accuracy is not known. For fast growing stocks like those on Georges Bank, any of the structures could be used equally well. However the **Workshop noted** that a hybrid of structures or methods for age estimation one stock could complicate the validation process. There 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.
- 3) *Different structures – different method for ageing:* The **Workshop recommended** that the left otolith on the side opposite the sulcus be the accepted standard for reading whole otoliths. 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 estimation 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.

- 4) *Edge determination*: The *Workshop noted* that age estimation is based on annuli formation and edge determination, and that the translucent zone is formed in the winter-spring period. Most discrepancies in age estimation 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.
- 5) *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.
- 6) *Light*: The *Workshop recommended* the use of reflected light for otoliths and transmitted light for scale impressions.
- 7) *Otolith storage*: Otoliths and scales should be stored dry. Thin sections should be permanently mounted on slides or Plexiglas 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. Permout should be used instead.
- 8) *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.
- 9) *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 has not shown much promise but is useful for some stock identification and growth rates studies.
- 10) *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 in young of the year fish.

Age validation

- 1) 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 for young fish. Following modal peaks of strong year-classes from research vessel surveys on the 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.
- 2) For the Georges Bank stock, the *Workshop recommended* that a validation study be carried out for the older age groups.

Preparation of reference collection

The Workshop noted the purpose of the reference collection is to use it 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.

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.

Training

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

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.

Quality Control

The Workshop discussed how often the reference collection should be read. Some ideas that were brought up included, 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 1 000 production otoliths). The Workshop recommended that before production age estimation begins, the following sequence should take place:

Step 1, perform a pre-calibration test by reading ~100 otoliths from the previous year's collection to 'warm up' and then use age bias plots and CVs 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 CVs.

The Workshop noted 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.

Bias and precision

The *Workshop recommended* that age bias plots and CVs were the most acceptable statistics to evaluate the results of monitoring, i.e. within reader drift and variability in age estimation. With respect to how much

bias is too much, it was agreed that the trend or tendency should be noted in terms of a deviation from a 1:1 line. A deviation of one year might be important or it might be insignificant. Also important is to look for bias in young fish or in abundant age classes. Based on past experience it was noted a bias is mostly observed in young and old fish.

The Workshop reviewed which CV reference level should be used to identify the threshold between acceptable and unacceptable degrees of precision among yellowtail flounder age readers. No one CV level was found to be acceptable for all species and it was noted to be 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) CVs 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, the Workshop reviewed how should age readers attempt to fix this problem. It was agreed that using age bias plots can help to determine where the bias is located. The *Workshop endorsed* the Beanlands (1997) Inter-Regional Ageing Workshop recommendations on this matter: 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.

How often and when to update the reference collection

It was agreed, 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 stated above) add these to the reference collection. In the case of otoliths stored in glycerine and used for the reference collection, it was agreed digital images should be taken.

Secondary age readers

The *Workshop concluded* that a second age reader is not necessary for Quality Control. It stretches the manpower resources, since one needs to wait for the second reader's verification and it is not clear what advantage there is in 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 estimation, 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.

Revision of Historical Age Readings (Archival Conversions)

The Workshop *concluded* that based on the whole vs sectioned otolith comparison carried out by age readers at NAFC, Grand Bank yellowtail flounder above 35 cm appear at risk of being under-aged. This may be the case for other stocks as well. The Workshop further noted that because 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 suggested* that the first approach would be sub-sampling of otoliths stratified by length, sex, area and season. If there is no confidence in the sub-sampling, the entire collection will have to be re-read as was the case for Scotia-Fundy haddock.

Summary of Recommendations

Age Reading Protocols

- 1) The *Workshop recommended* that the left otolith on the side opposite the sulcus be the accepted standard for reading whole otoliths. 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 young of the year fish.

Specific stocks

- 1) 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.
- 2) For the Georges Bank and the Grand Bank stocks, the *Workshop recommended* that a validation study be carried out for the older age groups.

Statistics

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

Reference Collection

- 1) 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.
- 2) The *Workshop recommended* that a minimum of 500 otoliths or scales be used for the reference collection.
- 3) The *Workshop recommended* a minimum of 100 otoliths or scales be used for the training session.
- 4) 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.

Calibration and production ageing

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 CVs 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 CVs.

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.

Conclusions

Slow growing coldwater species are often under aged when using the whole otolith method and investigations into age estimation using sectioned otoliths should be pursued. A comparative examination should be carried out in the manner described in this Workshop.

Future Considerations

The Workshop agreed that 1) OTC (oxytetracycline) marking during regular tagging studies should be encouraged and that a scrape of scales should be taken for age estimation and 2) that progress on implementation of above recommendations should be reviewed in 3 years.

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**Appendix I. Agenda for the Canada-United States
Yellowtail Flounder Age Reading Workshop
28–30 November 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*

0900 –1700 hrs:

- 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).
- 3) Discussion of standardization of terminology.
- 4) Discussion of standardization of age reading protocols.
- 5) Slide show on scales and otoliths – discussion topics:
 - 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.
- 7) Start outline of Workshop report.
- 8) Demonstration of equipment and techniques for age estimation yellowtail flounder.

Wednesday, November 29thConvener: *Jay Burnett*Rapporteur: *Heath Stone***0900 –1700 hrs:**

- 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.
- 3) Introduction to determining accuracy, precision, age validation and quality control in otolith age estimation.
- 4) Presentation by those labs ageing yellowtail flounder on studies related to Topic #3.
- 5) General discussion on current practices in accuracy, precision, age validation and quality control in otolith age estimation.
- 6) Continue outline of Workshop report.
- 7) Further demonstration of otolith preparation techniques if time is available.

Thursday, November 30thConvener: *Steve Campana*Rapporteur: *Karen Dwyer***0900 –1500 hrs:**

- 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.
- 5) Presentation of draft outline of the Workshop report.

Appendix II. Workshop Participants

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 St. John's harbour in the background.*

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Appendix III. Regional Reports on Biology and Age Reading of Yellowtail Flounder

ANNEX 1. Report by NAFC: Yellowtail Flounder Research on the Grand Bank

by

Stephen J. Walsh, Mick Veitch and Karen Dwyer

Northwest Atlantic Fisheries Center, St. John's, NL A1C 5X1

The Grand Bank stock, Northwest Atlantic Fisheries Organization (NAFO) Division 3LNO, is under quota regulation and because it straddles Canada's 360 km (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 tons 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 the NAFO Regulatory Area of Div. 3N, known as the Tail of the Bank.

Since 1973, the stock has been managed by regulating a total allowable catch (TAC), ranging from 7 000 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 Fisheries Commission in 1994. The fishery re-opened in 1998 with a precautionary TAC of 4 000 tons.

The following are comments and Figures regarding distribution and biology of yellowtail flounder on the Grand Bank.

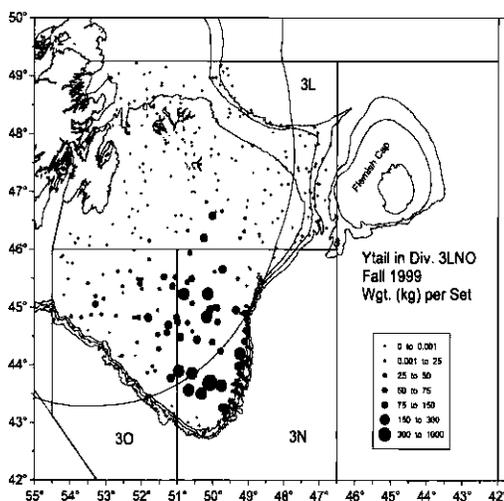


Fig. 1. Distribution.

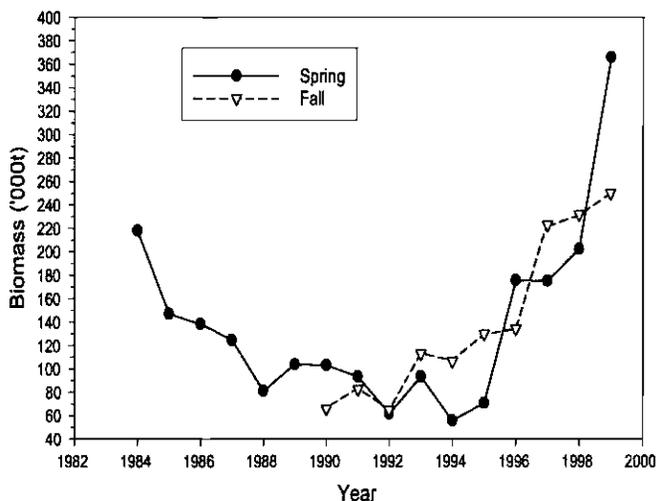


Fig. 2. Biomass

Factors affecting distribution

- Temperature range – 1.2 to 5.8°C
- Depth range 40–100 m
- Sandy bottom
- Spawning May to September

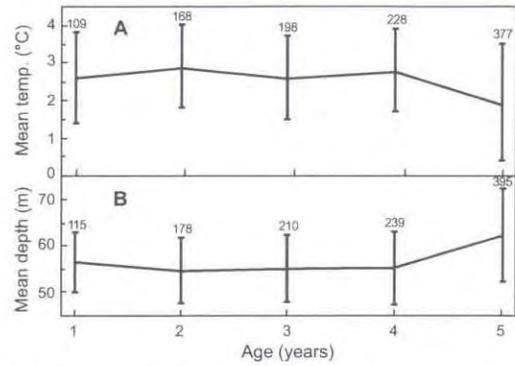


Fig. 3. Age related distribution by bottom temperature and depth.

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
- A January 1 birthday is not used

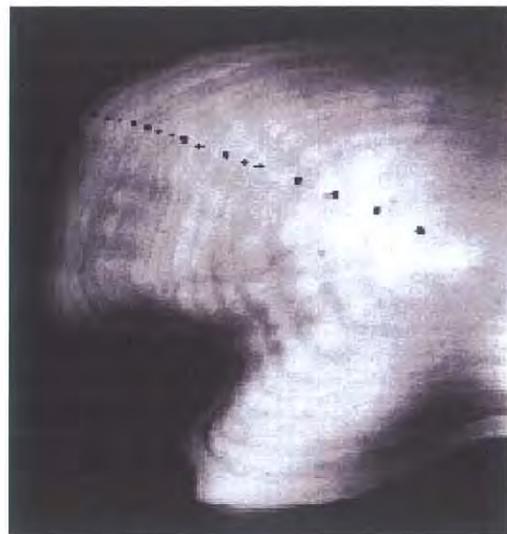


Fig. 4. A typical age reading.

Growth

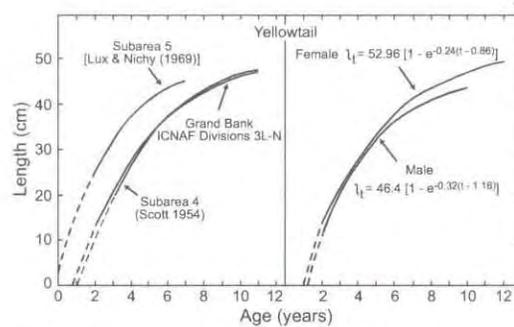
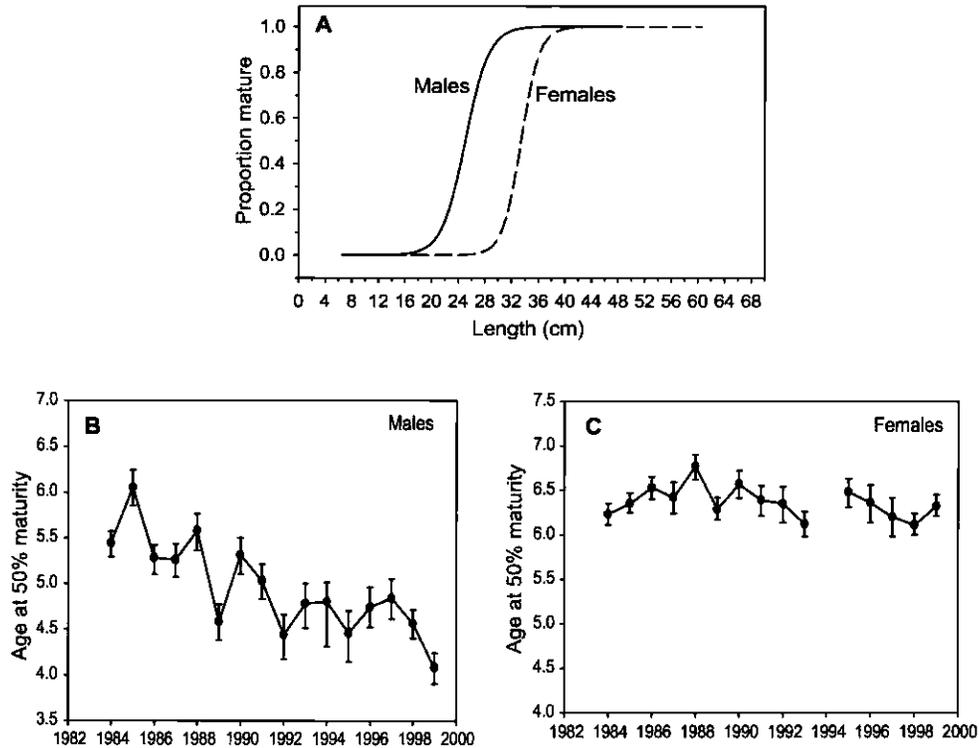


Fig. 5. Historic view of growth from Pitt (1974).

Maturity

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) as shown in Figures A, B and C.



Tagging Studies – information on age and growth

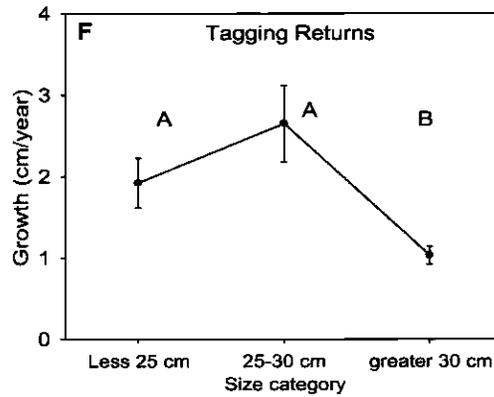
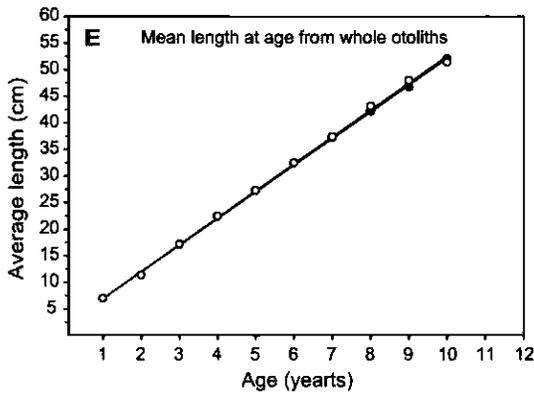
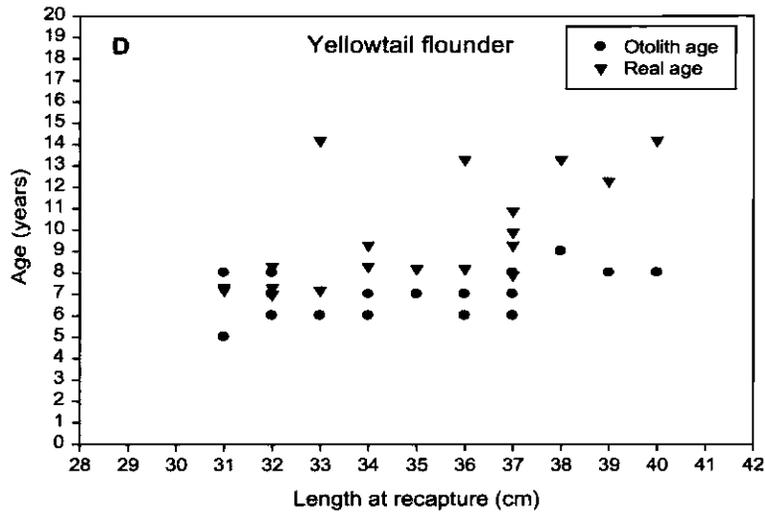
During the 1990–93 period tagging studies of juvenile yellowtail flounder on the southern Grand Bank took place (Morgan and Walsh, MS 1999). The fishery was under moratorium from 1994–98 and since it reopened several tags have been returned

Figure D, E and F show tagging returns indicate that yellowtail flounder may be older than we are currently ageing them and possibly growing slower than we have estimated.

We propose a fresh approach to investigating age and growth of yellowtail flounder, 1999 – onward be undertaken noting we have research otoliths from 1949 – present and commercial otoliths from 1965 – present.

In the old system up to 1999, the following were noted:

- One age reader reading whole otoliths
- Older ages were underestimated and hence,
- Possibly underestimating growth



In the new Approach for 2000 we have adopted a systematic approach to our underage estimation problem:

- Use two age readers
- Investigated various methods of preparation such as sectioning, staining and baking otoliths
- Compared age estimation from scales and otoliths
- Investigated ages from length frequency
- Investigated larval length frequencies for size at settlement
- Started a reference collection Age reading Workshop

References

MORGAN, M. J., and S. J. WALSH. MS 1999. An update of results of tagging experiments with juvenile yellowtail flounder in NAFO Divisions 3LNO. *NAFO SCR Doc.*, No. 23, Serial No. 4074, 8 p.

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

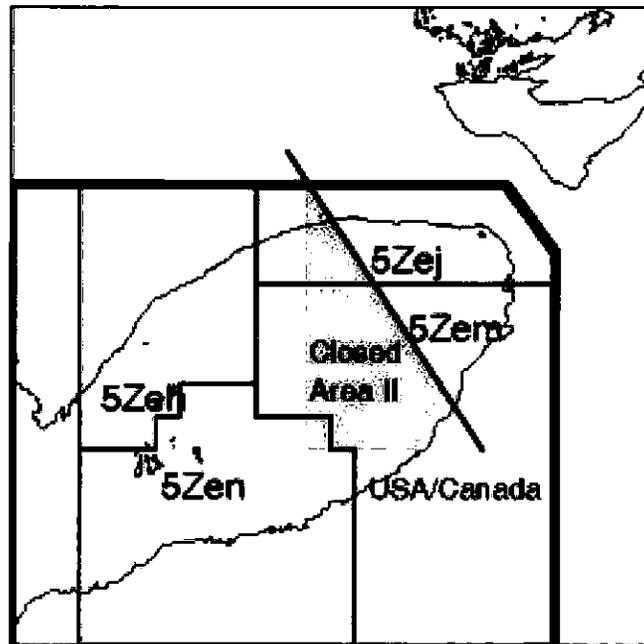
Annex 2. Report by SABS: Georges Bank Yellowtail Flounder

by

Heath Stone and Peter Perley

St. Andrews Biological Station, St. Andrews, New Brunswick

- 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 1930s).
- Currently managed as a transboundary resource, with 5Zhjmn (see Fig. below) 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-1990s, 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.

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)(see Fig. 1 below).
- 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).

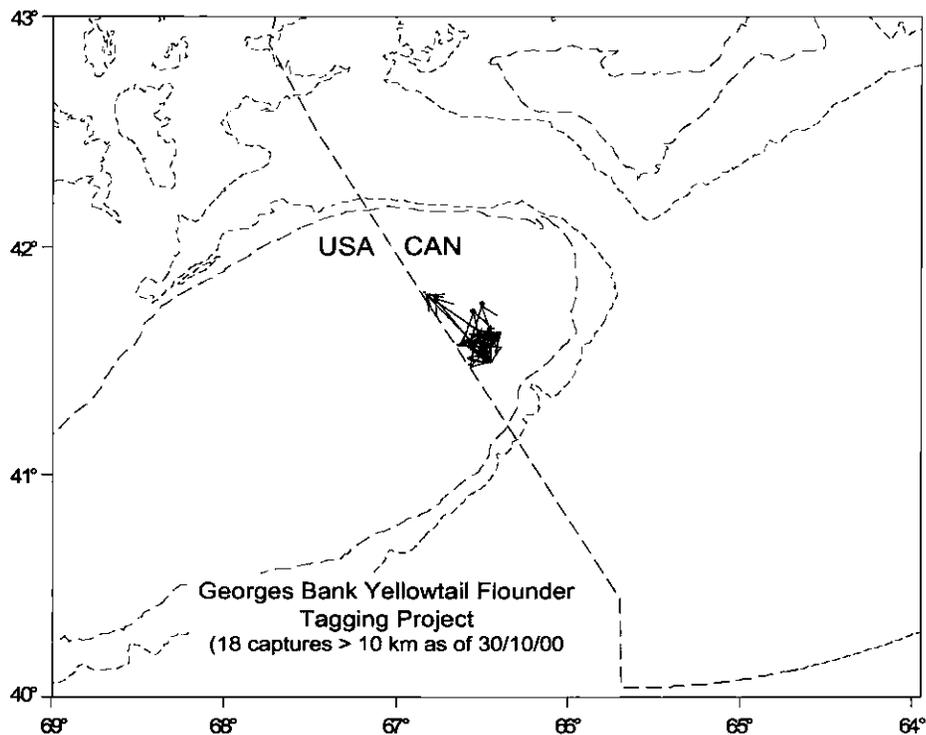


Fig. 1. Release and recapture plot.

Maturity (based on Royce *et al.*, 1959)

Females: 50% mature at 32 cm TL, 90% at 40 cm 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 Georges Bank yellowtail flounder was conducted by Lux and Nichy (1969) using scales (see Fig. 2 below).
- Good documentation of first annulus formation and growth through age two.
- Partial validation of ageing 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 autumn 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 ageing 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.

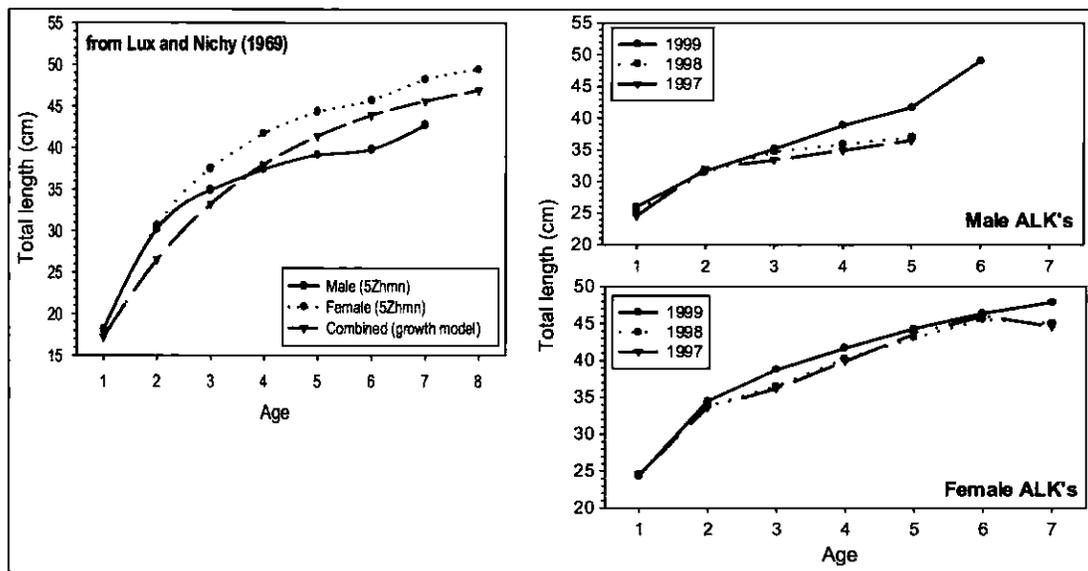


Fig. 2. Age and growth information.

Summary

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

Several Issues to Address:

- The Lux and Nichy (1969) 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 Georges Bank 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 Canadian 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 1989–90 and an increase from 1999–2000 (Change in age reader? Spatial/temporal variability in sampling? Change in growth rates?).

Ageing 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.

Sampling and Storage: RV Survey Collections

- Inventory at SABS includes over 5 000 otolith pairs from Canadian RV survey collections (1987–91; 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	0
1994	N200	–	–	–	0
1995	N216	–	–	–	0
1996	N237	0	335	272	607
1997	N254	7	409	431	847
1998	N773	5	261	345	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 flounder 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

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.

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

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. Rep.*, **NMFS 72**: 119–124.
- 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.

ANNEX 3. Report by MDMF: Sampling of Cape Cod Stock Yellowtail Flounder by the State of Massachusetts Trawl Survey

by

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Massachusetts Division of Marine Fisheries (MDMF) has conducted a bottom trawl survey in state territorial waters every May and September since 1978 (Fig. 1). 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 (Fig. 1). The Massachusetts survey fishes with a ¾ size North Atlantic type two seam otter trawl (11.9 m headrope – 15.5 m footrope) rigged with a 19.2 m chain sweep with 7.6cm rubber discs.

On average, greater than 1 600 yellowtail flounder are captured each spring and yellowtail flounder are present at greater than 40 stations. In autumn, greater than 1 000 yellowtail flounder are captured at more than 30 stations. The depth stratum 36.7–54.9 m produces the greatest yellowtail flounder catches in each of the three survey regions.

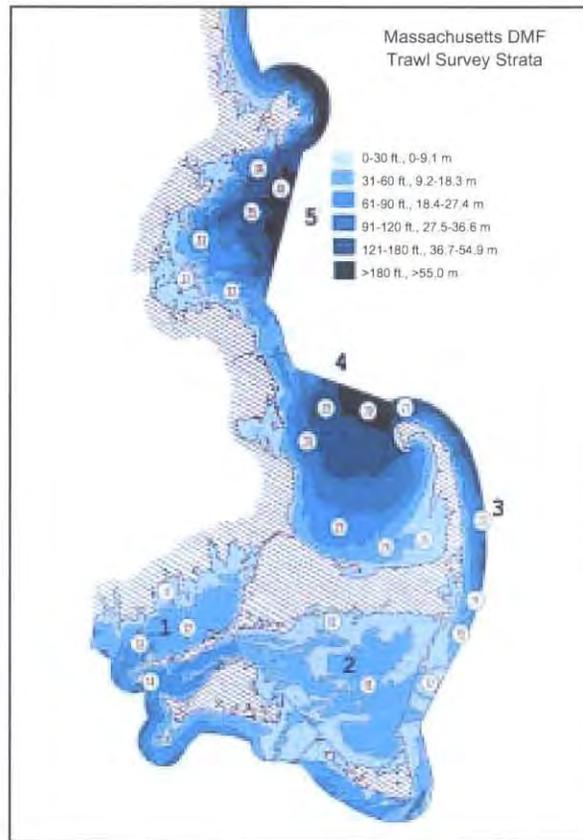


Fig. 1. Massachusetts CMF trawl survey strata.

Stratum 35 (36.7-54.9 m) 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 flounder population metrics produced from the Massachusetts survey (Fig. 2).

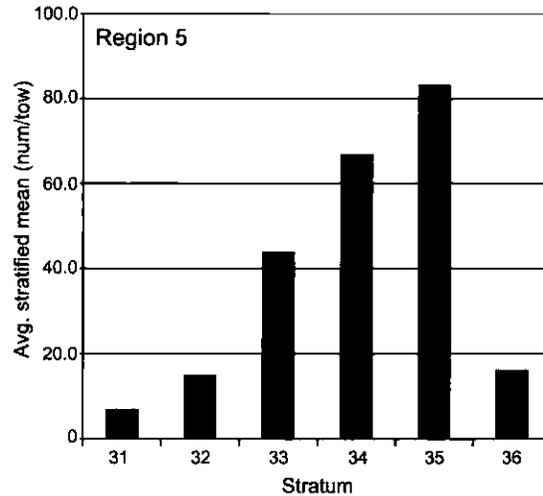


Fig. 2. Mean number per two in Region 5.

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 (Fig. 3). The largest catches of juveniles occurred at 4–8°C in spring and 8–11°C in autumn (Fig. 4), while adult temperature preferences appeared similar with greatest spring catches at 4–9°C and 8–11°C in autumn (Johnson, *et al.*, MS 1999).

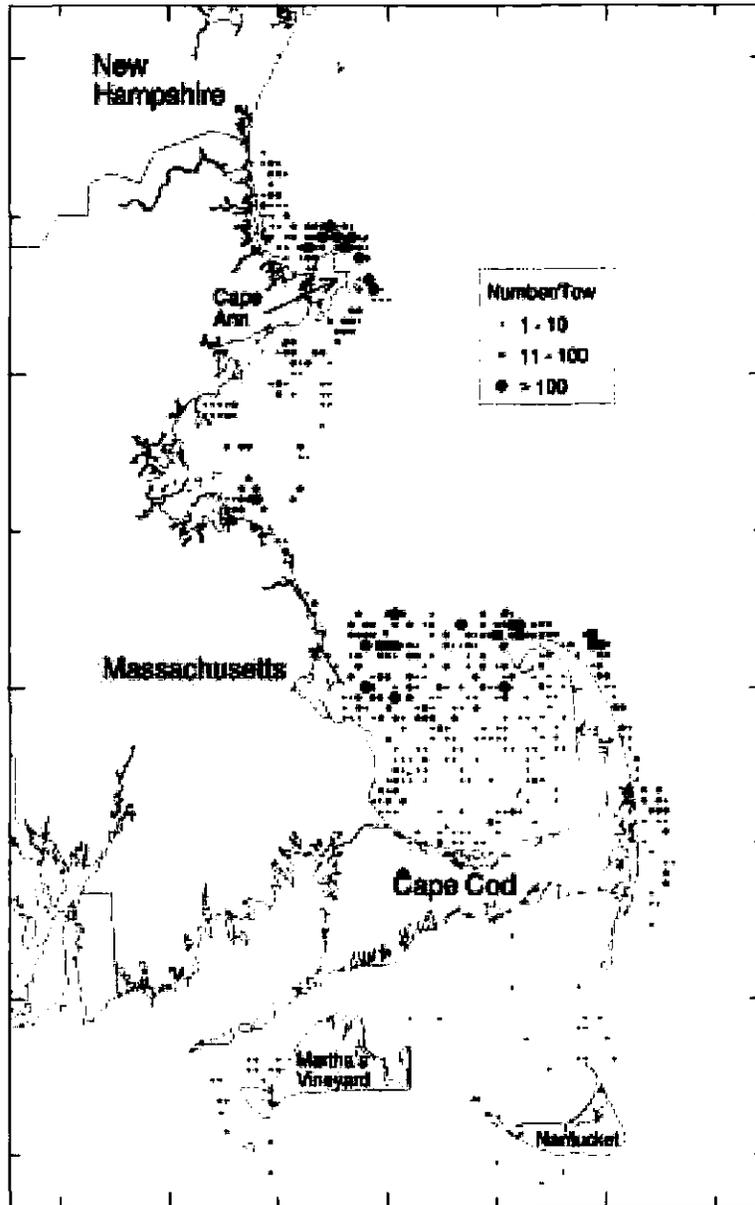


Fig. 3. Yellowtail flounder – Juveniles (≤ 26 cm)
Spring (1978–2001)
Massachusetts inshore trawl survey.
Fish Caught/station (excluding 0 catch): Mean: 18 Min: 1 Max: 550
Length (cm): Min: 3 Max: 26

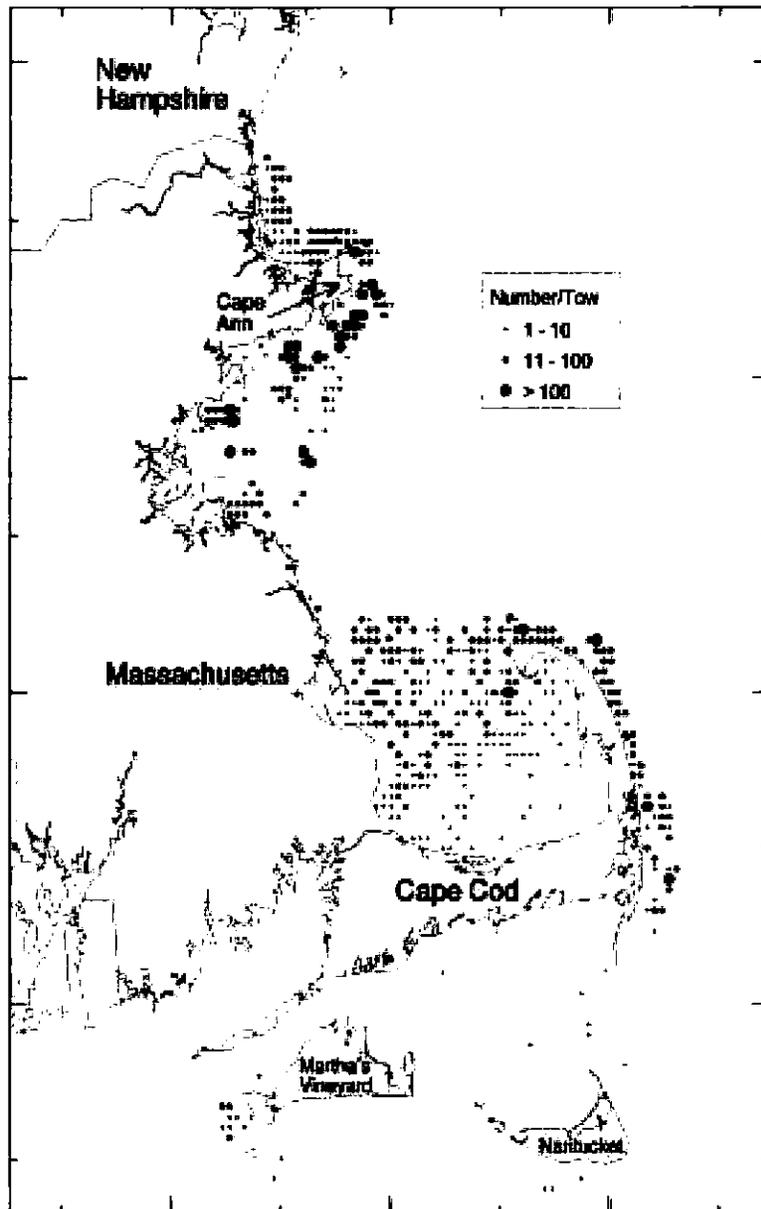


Fig. 4. Yellowtail flounder – Adults (≥ 27 cm)
Spring (1978–2001)
Massachusetts inshore trawl survey
Fish Caught/station (excluding 0 catch): Mean: 18 Min: 1 Max: 550
Length (cm): Min: 3 Max: 26

Recent MDMF efforts to differentiate yellowtail flounder 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 flounder catch in stratum 35 was 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 (Fig. 5) indicate that the commercial catch is mostly (93%) composed of ages 2–4 (Cadrin, MS 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.

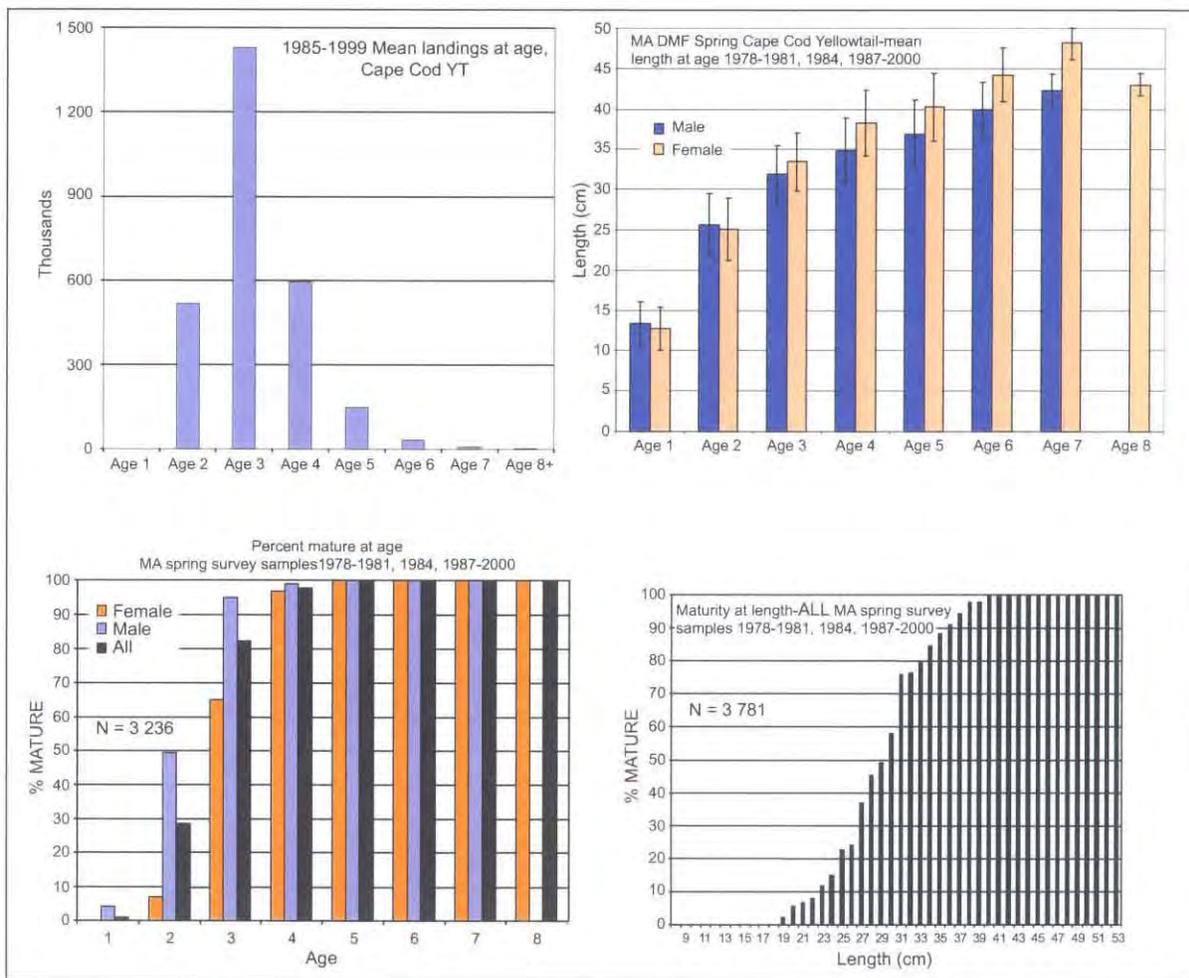


Fig. 5. VPA results.

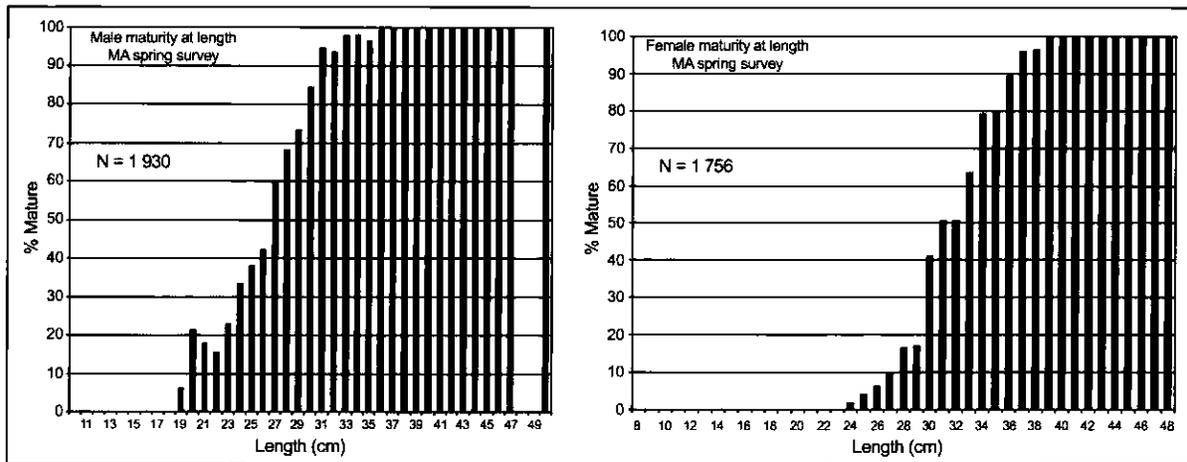


Fig. 5. VPA results.

References

- CADRIN, S. X. MS 2000. Cape Cod yellowtail flounder. *In*: Assessment of 11 Northeast groundfish stocks through 1999. *NEFSC Ref. Doc.*, No. 00-05: 83-98.
- JOHNSON, D. L., W. W. MORSE, P. L. BERRIEN, and J. J. VITALIANO. MS 1999. Essential fish habitat source document: Yellowtail flounder, *Limanda ferruginea*, Life history and habitat characteristics. *NOAA Tech. Memo.*, NMFS-NE-140, 29 p.

**ANNEX 4. Report of NEFSC: 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
National Marine Fisheries Service, Woods Hole, Massachusetts

Yellowtail Flounder Stock Structure in Subareas 5 and 6

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

Distribution by Depth

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

Distribution by Temperature

- 2–12°C (0.5–15°C)
- Spawning March–August (May–June)
- Murawski and Finn 1988

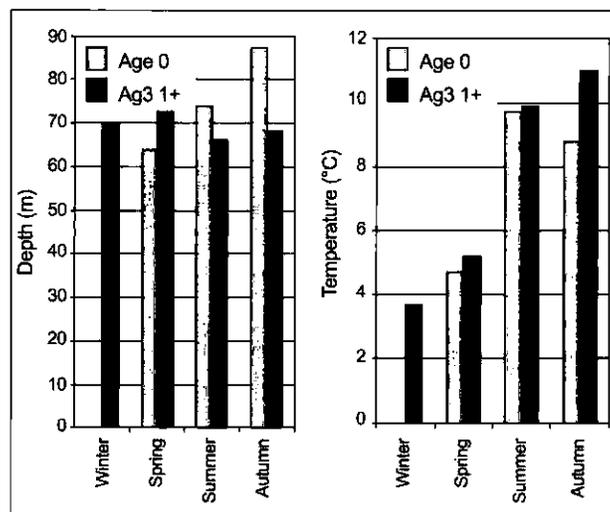


Fig. 1. Distribution by depth and temperature.

Growth

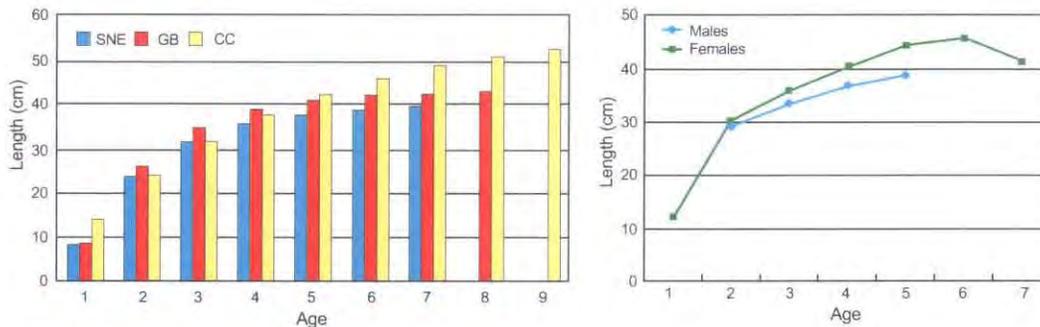


Fig. 2. Growth by region and sex.

Yellowtail Maturation – Males

Stock	L_{50}
SNE	19.6 cm
GB	21.4 cm
CC	26.8 cm

Yellowtail Maturation – Females

L_{50}
25.5 cm
25.8 cm
29.7 cm

Age Validation Studies

- 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

Sampling Protocols

- 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 ageing

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.
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**Appendix IV. 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)	Div. 3LNO	1965-present	1949-present (Spring.) 1985-present (Autumn.)	1965-present	1949-present	Otoliths scales (1999-2000)
St. Pierre Bank (NAFC)	Subdiv. 3Ps	-	1972-present	-	1972-present	otoliths
S. Gulf of St. Lawrence (GFC)	Div. 4T	1985-88, 1992, 1995-2000	1971-present	-	1972-1982	otoliths
Scotian Shelf (BIO)	Div. 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)	SA 5&6	1957-present	1963-present (Autumn.) 1968-present (Spring.) 1992-present (Winter.)	1965-present	1963-present	scales
Georges Bank - Canada (SABS)	Div. 5Z hjm	1994-present	1987-present	1995-present	1987-1991, 1996-present	Otoliths scales (2000)

Appendix V. Age Reading Comparisons At Various Institutes

Annex 1. Age Reading Comparisons at NAFC

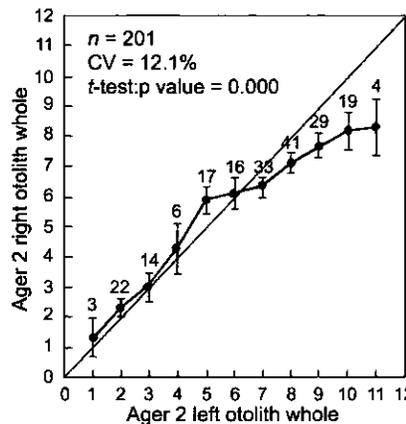
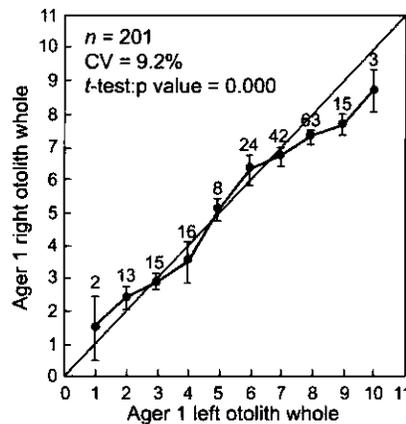
by

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

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 (Campana *et al.*, 1995)..

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 reading 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.

References

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- CAMPANA, S. E., M. C. ANNAND, and J. I. MCMILLAN. 1995. Graphical and statistical methods for determining the consistency of age determinations. *Trans. Am. Fish. Soc.*, **124**: 131–138.
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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.

Annex 2. 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 Agrifoods
Government of Newfoundland and Labrador

The Provincial Government of Newfoundland is currently using techniques and standards that may assist in the ageing 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 μ 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 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 (100× 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:

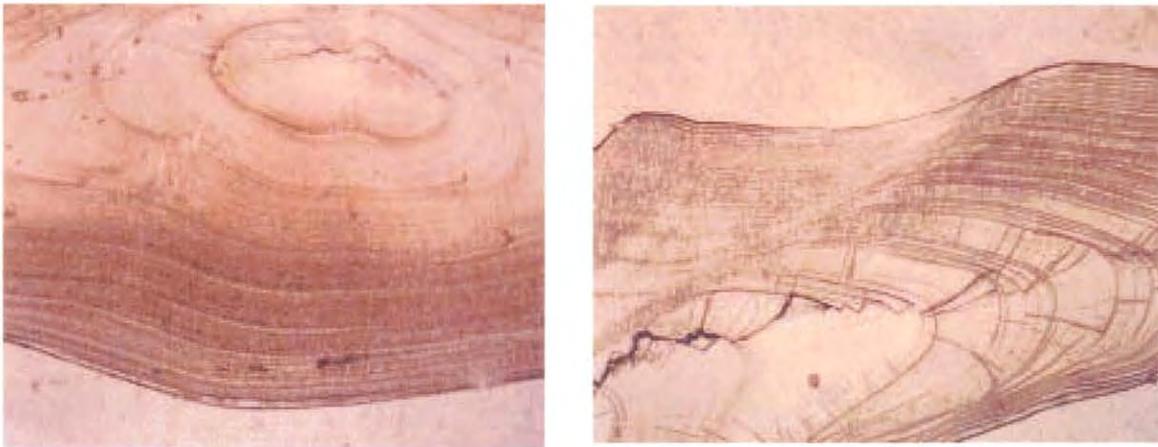
Dr John Casselman
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Aquatic Ecosystems Science Section
Glenora Fisheries Station
R.R. 4, Picton Ontario
K0K 2T0

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 Fisheries Station, Ontario Ministry of Natural Resources, Picton, Ontario, Canada, 90 p.



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



Annex 3. 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, New Brunswick

- 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					Total
	2	3	4	5	6	
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 otoliths 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							Total
	2	3	4	5	6	7		
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							Total
	2	3	4	5	6	7		
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

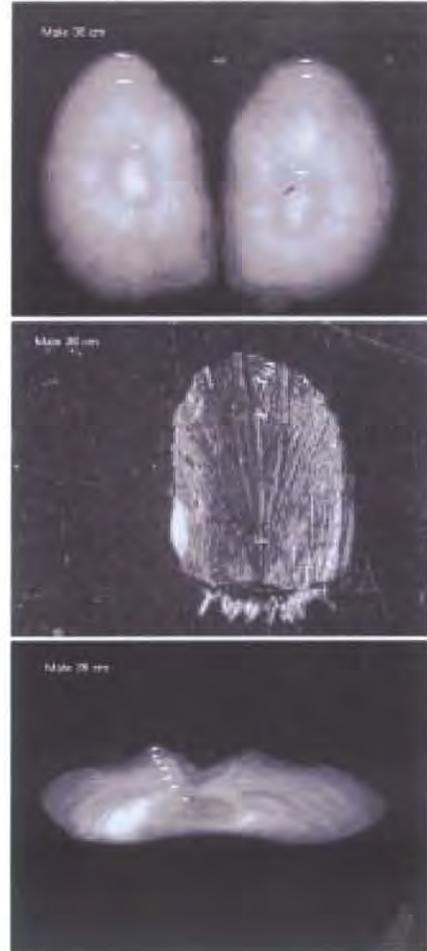
(52% Agreement)

Comparison of Otolith Cross-Section Age vs Scale Age

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

Appendix VI. Accuracy, Precision and Age Validation

Accuracy, Precision and Age Validation at NAFC

by

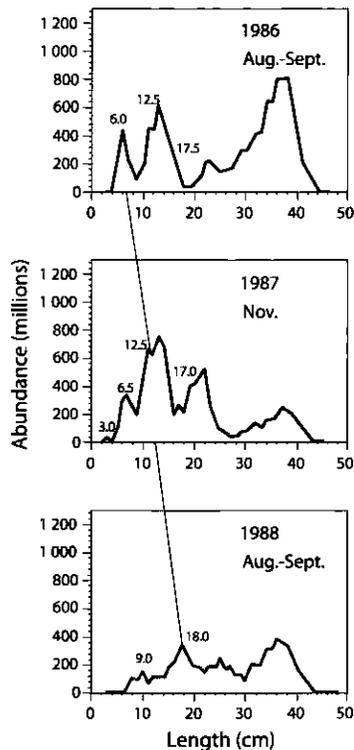
Karen Dwyer, Steve Walsh and Mick Veitch

Northwest Atlantic Fisheries Centre, St. John's, Newfoundland

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 0.75 mm × 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.

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 1985–94 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 1986–88. Modes from these years revealed that demersal 0-group yellowtail flounder were about 3–4 cm,

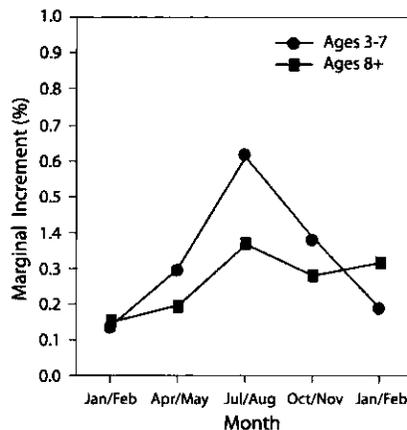


indicating metamorphosis at about 3–3.5 cm. The one-year-old mode was strong, and the size of fish of this age was estimated to be approximately 7 cm in males and females. The size of age 2-year-old fish was estimated to be 13 cm and 14 cm in males and females, respectively.

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 flounder verify that under optimum growing conditions, yellowtail flounder 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

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Appendix VII. Annotated Images From Each Region

Grand Bank Otoliths – fish captured in autumn, 1999.



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



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



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



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



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

Southern Gulf Otoliths – fish captured in September 2000



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



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



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

Scotian Shelf Otoliths – fish captured in July, 1979.



Fig. 3A. Age estimated by panel for whole otolith 7 years (Length and sex of fish unknown).



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



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



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

Canadian Georges Bank Otoliths/Scales – fish captured in October, 2000.



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



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



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

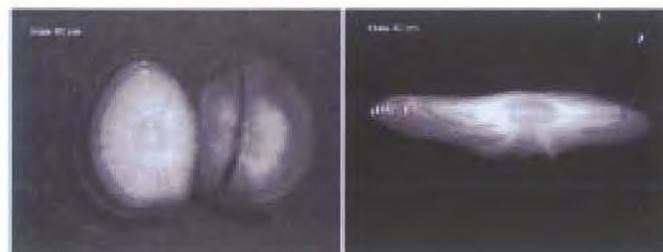


Fig. 4D. Age estimated by panel for whole otolith 4 years; for section 7 years.

United States Georges Bank Otoliths – fish captured in autumn, 2000

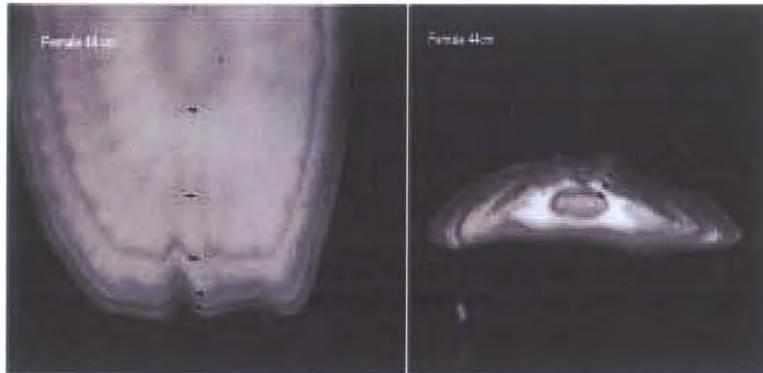


Fig. 5A. Age estimated by panel for whole otolith 5 years; for section 5 years.



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

NOTICE

Precautionary Approach Workshop

Hosted by the Scientific Council of the Northwest Atlantic Fisheries Organization

31 March – 4 April 2003
Delta Hotel, St. John's, Canada

Since 1997, Scientific Council has dedicated considerable resources towards the development of a framework for implementing the Precautionary Approach. A proposed general framework was developed in 1997 to include limit and buffer reference points for fishing mortality and biomass. A Scientific Council Workshop on the Precautionary Approach to Fisheries Management was held in March 1998 to review methodology and propose reference points for some stocks. The Scientific Council proposed that this second Workshop address reference points for additional priority stocks, and the basis for reference points that have already been identified by Scientific Council should also be reviewed.

Terms of Reference for the Workshop are:

- Review the basis for existing PA reference points.
- Determine appropriate methodology to calculate reference points for data-limited stocks.
- Develop or revise additional reference points for the following stocks:

Greenland halibut in SA 2 and Div. 3LKMNO

American plaice in Div. 3LNO

Cod in Div. 3NO

Yellowtail flounder in Div. 3LNO

Redfish in Div. 3M

Cod in Div. 3M

Shrimp in SA 0 and 1

- Provide guidance to Designated Experts for calculating PA reference points for all remaining stocks for which sufficient data exist.

The Workshop will develop reference points for data-rich stocks, based on results from Analytical Models. For data-limited stocks, proxy reference points will be developed based on survey data using methodology developed to address proxy reference points.

The Joint Fisheries Commission/Scientific Council Working Group on the Precautionary Approach and the Working Group of Technical Experts have identified several managerial areas of concern with the specification of the Precautionary Approach framework originally proposed by the Scientific Council. In particular, the Working Group questioned the requirement for a linear reduction in F between B_{target} and B_{lim} , and a fishery closure when the stock is below B_{lim} , and the suitability of F_{msy} as a limit reference point. Therefore, an additional term of reference is proposed for the Scientific Council Workshop:

- Re-examine the PA framework as described in *NAFO SCS Doc. 97/12* and develop more precise definitions of terms and concepts consistent with the role of scientists and managers as agreed in *NAFO FC Doc. 98/2*.

A small Study Group working by correspondence in advance of the proposed Scientific Council Workshop may address this last term of reference.

NOTICE

Workshop on Mapping and Geostatistical Methods for Fisheries Stock Assessment

Hosted by the Scientific Council of the Northwest Atlantic Fisheries Organization (NAFO)

10–12 September 2003
Holiday Inn, Dartmouth, Canada

The Scientific Council of NAFO is pleased to announce this Workshop to be held in conjunction with the NAFO Annual Meeting in Canada in September 2003. The workshop will be convened by L. Hendrikson (National Marine Fisheries Service – USA) and D. W. Kulka (Fisheries and Oceans – Canada) and organized by the NAFO Secretariat.

The purpose of the Workshop is two fold:

- a) To introduce Scientific Council fisheries scientists, using practical demonstrations relevant to NAFO issues, to spatial techniques that can be applied to survey and environmental data to solve fisheries problems.
- b) To provide Scientific Council members with enough background that they can interpret GIS analyses.

GIS is a broad field and the Workshop can only touch upon some of the aspects of spatial analysis. It will focus on techniques that will be most useful to the user group. The Workshop will:

- show how the raw data (set attributes) can be effectively Visualized (mapping techniques), progressing through Point to Surface Transformation (i.e. methods such as Contouring, Voronoi, Potential Mapping, Kriging that produce the continuous surfaces required to facilitate spatial modeling),
- to Overlay Modeling, and geostatistics (outside expertise is required to deal with this aspect).
- evaluate mixed species interactions within the Precautionary Approach framework.

The workshop will be structured to introduce NAFO Scientific Council participants to a subset of techniques and concepts relevant to their work. Examples and demonstrations will use real data from the Grand Banks and Flemish Cap. Where appropriate underlying theory and procedures of geostatistics will be elaborated to facilitate the understanding of GIS analyses.

Scientific Council agreed that the proposed workshop is important to the work of Scientific Council. The Council scheduled the workshop to be held in conjunction with the Annual Meeting in 2003, during 10–12 September 2003.

Information for Preparing Manuscripts for NAFO Scientific Publications

Introduction

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

Content of Manuscript

Title page

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

Abstract

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

Text

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

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

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

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

The **Discussion** should give the main contributions from the study, with appropriate interpretation of the results focussing on the problem or hypothesis. Compare with those of other authors. Speculation should be limited to what can be supported with reasonable evidence. In the case of short papers, it may be useful to combine Results and Discussion to avoid repetition.

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

The **References** represents the list of references cited in the text listed alphabetically. Good judgment should be used in the selection of references, which should be restricted largely to significant published literature. Unpublished data and documents, manuscripts in preparation, and manuscripts awaiting acceptance to other journals may be noted in the text as unpublished data or personal communications, with full contact addresses.

Literature references cited in the text must be by author's surname and year of publication, e.g. (Collins, 1960). The surnames of two authors may be used in a citation, but, for more than two authors the citation should be (Collins *et al.*, 1960). The citation of mimeographed manuscript reports and meeting documents should contain the abbreviation "MS", e.g. (Collins *et al.*, MS 1960). All papers referred to in the text must be cited in the References alphabetically by the first author's surname and initials, followed by the initials and surnames of other authors, year of publication, full title of the paper, name of the periodical, volume and/or number, and range of pages. Abbreviations of periodicals should, if possible, follow the "World List of Aquatic Sciences and Fisheries Serials Titles", published periodically by FAO (Food and Agriculture Organization of the United Nations). References to monographs should, in addition to the author(s), year and title, contain the name and place of the publisher and the number of pages in the volume. Reference to a paper in a book containing a collection of papers should also contain the page range of the paper, name(s) of editor(s), and actual title of the book. The accuracy of all references and their correspondence with text citations is the responsibility of the author.

Comments on Tables and Figures

All Tables and Figures must be mentioned or discussed in the text. Tables and Figures must be numbered consecutively in arabic numerals, which correspond with the order of presentation in the text. The required position of the Tables and Figures in the text should be indicated in the left margin of the relevant page. Place the originals of Tables and Figures after the list of references.

Tables. Note a well constructed Table can eliminate elaborate text descriptions. Each Table should be carefully constructed to be easily read and understood. Each column and row must be concisely headed, ensuring relevant units of the values are given (usually within parentheses). Each Table should have a complete but concise descriptive heading, and should be on a separate sheets.

Figures. Note any reference to geographic areas relevant to the study should be shown in a Figure (or map) form giving coordinates. These and illustrations and photographs can eliminate elaborate text descriptions.

Each Figure should be carefully constructed and labelled to be easily read and understood. Each vertical and horizontal axis (e.g. x and y axes on a graph or latitude and longitudes on a map) must have a concise header with relevant units (usually within parentheses). Each Figure would have a complete but concise descriptive heading, and should be on a separate sheet.

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

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

Manuscript Submission

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

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

computer electronic form. Coloured Tables and Figures are now accepted.

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

The Secretariat may request alternative formats as publication technologies develop.

Journal of Northwest Atlantic Fishery Science

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

Such manuscripts are considered for publication with the understanding that the content is unpublished and is not being submitted elsewhere for publication. Each manuscript is assigned to an Associate Editor of the Journals Editorial Board, for scientific editing. Papers are normally sent by the Associate Editors to two referees for appraisal regarding its suitability as a primary article.

NAFO Scientific Council Studies

The Studies publishes papers which are of topical interest and importance to the current and future activities of the Scientific Council, but which are not considered to be sufficiently high quality to meet the standards for primary publication in the Journal. Such papers have usually been presented as research documents at Scientific Council meetings and nominated for publication by the Standing Committee on Publications. These manuscripts are not normally refereed but undergo critical scrutiny by the Studies editor and by an expert familiar with the subject matter selected from the Journal editorial board.

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

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Northwest Atlantic Fisheries Organization
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Scientific Publications of the Northwest Atlantic Fisheries Organization

Journal of Northwest Atlantic Fishery Science

The Journal provides an international forum for the primary publication of original research papers on fisheries science in the Northwest Atlantic, with emphasis on environmental, biological, ecological and fishery aspects of the living marine resources and ecosystems. (Scientific publications during ICNAF times are available at the Secretariat).

- Vol. 1 – Miscellaneous papers, (10), December 1980, 112 pp.
- Vol. 2 – Miscellaneous papers, (10), October 1981, 76 pp.
- Vol. 3, No. 1, 2 – Miscellaneous papers, (17), May and December 1982, 180 pp.
- Vol. 4 – Special issue *Guide to the Early Stages of Marine Fishes Occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf*, July 1983, 424 pp.
- Vol. 5, No. 1, 2 – Miscellaneous papers, (26), January and November 1984, 224 pp.
- Vol. 6, No. 1, 2 – Miscellaneous papers, (17), June and December 1985, 179 pp.
- Vol. 7, No. 1, 2 – Miscellaneous papers, (18), December 1986 and December 1987, 177 pp.
- Vol. 8 – Miscellaneous papers, (7), December 1988, 88 pp.
- Vol. 9 – Miscellaneous papers, (13), September and December 1989, 159 pp.
- Vol. 10 – Special issue, (1), *The Delimitation of Fishing Areas in the Northwest Atlantic*, December 1990, 57 pp.
- Vol. 11 – Miscellaneous papers, (7), February 1991, 80 pp.
- Vol. 12 – Miscellaneous papers, (7), January 1992, 84 pp.
- Vol. 13 – Miscellaneous papers, (7), December 1992, 114 pp.
- Vol. 14 – Symposium papers, (12), on *Changes in Biomass, Production and Species Composition of the Fish Populations in the Northwest Atlantic over the Last 30 Years, and Their Possible Causes*, December 1992, 160 pp.
- Vol. 15 – Special issue, (1), *Decapod Crustacean Larvae from Ungava Bay*, December 1993, 170 pp.
- Vol. 16 – Miscellaneous papers, (7), July 1994, 100 pp.
- Vol. 17 – Miscellaneous papers, (6), October 1994, 78 pp.
- Vol. 18 – Miscellaneous papers, (6) (1 Note), April 1996, 115 pp.
- Vol. 19 – Symposium papers, (11), on *Gear Selectivity/Technical Interactions in Mixed Species Fisheries*, September 1996, 145 pp.
- Vol. 20 – Special issue, (1), *North Atlantic Fishery Management Systems: A Comparison of Management Methods and Resource Trends*, September 1996, 143 pp.
- Vol. 21 – Miscellaneous papers, (5), April 1997, 83 pp.
- Vol. 22 – Symposium papers, (25) (1 Note), on *The Role of Marine Mammals in the Ecosystem*, December 1997, 387 pp.
- Vol. 23 – Symposium papers, (16), *What Future for Capture Fisheries*, October 1998, 277 pp.
- Vol. 24 – Miscellaneous papers, (4), November, 1998, 97 pp.
- Vol. 25 – Symposium papers, (17), (2 Notes), on *Variations in Maturation, Growth, Condition and Spawning Stock Biomass Production in Groundfish*, October 1999, 233 pp.
- Vol. 26 – Miscellaneous papers, (6), December 2000, 145 pp.
- Vol. 27 – Symposium papers (22) (1 Note), *Pandalid Shrimp Fisheries – Science and Management at the Millennium*, December 2000, 289 pp.
- Vol. 28 – Special issue, (1), *A Review of the Cod Fisheries at Greenland, 1910–1995*, December 2000, 121 pp.
- Vol. 29 – Miscellaneous papers, (5), December, 2001, 99 pp.

NAFO Scientific Council Studies

This publication includes papers of topical interest and importance to the current and future activities of the Scientific Council.

- No. 1 – Miscellaneous papers, (11), March 1981, 101 pp.
- No. 2 – Manual on *Groundfish Surveys*, December 1981, 56 pp.
- No. 3 – Miscellaneous papers, (8), April 1982, 82 pp.
- No. 4 – Special Session papers, (12), on *Remote-Sensing Applications to Fishery Science*, September 1982, 98 pp.

NAFO Scientific Council Studies (Continued)

- No. 5 – Symposium papers, (12), on *Environmental Conditions in 1970–79*, December 1982, 114 pp.
- No. 6 – Miscellaneous papers, (8), December 1983, 104 pp.
- No. 7 – Miscellaneous papers, (9), August 1984, 98 pp.
- No. 8 – Miscellaneous papers, (12), April 1985, 96 pp.
- No. 9 – Special Session papers, (17), on *Squids*, November 1985, 180 pp.
- No. 10 – Miscellaneous papers, (9), August 1986, 112 pp.
- No. 11 – Miscellaneous papers, (11), March 1987, 127 pp.
- No. 12 – Miscellaneous papers, (8), March 1988, 90 pp.
- No. 13 – Miscellaneous papers, (5), November 1989, 82 pp.
- No. 14 – Miscellaneous papers, (6), May 1990, 74 pp.
- No. 15 – Miscellaneous papers, (7), May 1991, 68 pp.
- No. 16 – Special Session papers, (22), on *Management Under Uncertainties*, November 1991, 190 pp.
- No. 17 – Workbook on *Introduction to Sequential Population Analysis*, February 1993, 98 pp.
- No. 18 – Symposium papers, (18), on *Changes in Abundance and Biology of Cod Stocks and Their Possible Causes*, July 1993, 110 pp.
- No. 19 – Miscellaneous papers, (8), October 1993, 98 pp.
- No. 20 – Miscellaneous papers, (7), February 1994, 114 pp.
- No. 21 – Collections of Papers, (10), Related to *Northern Cod and Seals in NAFO Divisions 2J and 3KL*, December 1994, 165 pp.
- No. 22 – Miscellaneous papers, (6), May 1995, 95 pp.
- No. 23 – Miscellaneous papers, (5), September 1995, 95 pp.
- No. 24 – Symposium papers, (12), on *Impact of Anomalous Oceanographic Conditions at the Beginning of the 1990s in the Northwest Atlantic on the Distribution and Behaviour of Marine Life*, September 1994, 155 pp.
- No. 25 – Collection of Papers, (5), *Flemish Cap Selected Environmental and Other Papers*, July 1996, 91 pp.
- No. 26 – Selected Papers, (11), (2 Notes), on *Harp and Hooded Seals*, December 1996, 129 pp.
- No. 27 – Miscellaneous papers, (5), (1 Note), December 1996, 81 pp.
- No. 28 – Special Session papers, (6), on *Assessment of Groundfish Stocks Based on Bottom Trawl Survey Results*, December 1996, 105 pp.
- No. 29 – Selected Papers, (11), *Selected Studies Related to Assessment of Cod in NAFO Divisions 2J+3KL*, May 1997, 125 pp.
- No. 30 – Miscellaneous papers, (9), December 1997, 117 pp.
- No. 31 – Miscellaneous papers, (8), December 1998, 165 pp.
- No. 32 – Miscellaneous papers, (8), April 1999, 133 pp.
- No. 33 – Miscellaneous papers, (7), May 2000, 135 pp.
- No. 34 – Miscellaneous papers, (3), October, 2001, 91 pp.

NAFO Scientific Council Reports

This publication contains reports of Scientific Council Meetings held through each year since NAFO replaced ICNAF. (The comparable publication during ICNAF was called the *Redbook*).

- 1980 – Reports of seven meetings in 1979 and 1980, Published December 1980, 190 pp.
- 1981 – Reports of four meetings in 1981, Published December 1981, 148 pp.
- 1982 – Reports of two meetings in 1982, Published December 1982, 110 pp.
- 1983 – Reports of three meetings in 1983, Published December 1983, 152 pp.
- 1984 – Reports of three meetings in 1984, Published December 1984, 126 pp.
- 1985 – Reports of three meetings in 1985, Published December 1985, 146 pp.
- 1986 – Reports of three meetings in 1986, Published December 1986, 156 pp.
- 1987 – Reports of three meetings in 1987, Published December 1987, 138 pp.
- 1988 – Reports of two meetings in 1988, Published December 1988, 150 pp.
- 1989 – Reports of two meetings in 1989, Published December 1989, 180 pp.
- 1990 – Reports of two meetings in 1990, Published December 1990, 188 pp.
- 1991 – Reports of two meetings in 1991, Published December 1991, 164 pp.
- 1992 – Reports of four meetings in 1992, Published December 1992, 212 pp.
- 1993 – Reports of three meetings in 1993, Published January 1994, 234 pp.
- 1994 – Reports of four meetings in 1994, Published January 1995, 234 pp.

NAFO Scientific Council Reports (Continued)

- 1994 – Reports of four meetings in 1994. Published January 1995, 234 pp.
- 1995 – Reports of three meetings in 1995. Published January 1996, 244 pp.
- 1996 – Reports of three meetings in 1996. Published January 1997, 226 pp.
- 1997 – Reports of three meetings in 1997. Published January 1998, 274 pp.
- 1998 – Reports of three meetings in 1998. Published January 1999, 257 pp.
- 1999 – Report of four meetings in 1999. Published January 2000, 327 pp.
- 2000 – Report of four meetings in 2000. Published January 2001, 303 pp.
- 2001 – Report of three meetings in 2001. Published January 2002, 339 pp.
- 2002 – Report of three meetings in 2002. Published January 2003, 323 pp.

NAFO Statistical Bulletin

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

- Vol. 29 – Fishery statistics for 1979, Originally published July 1981; revised edition published November 1984, 290 pp.
- Vol. 30 – Fishery statistics for 1980. Originally published August 1982; revised edition published October 1984, 280 pp.
- Vol. 31 – Fishery statistics for 1981, Originally published September 1983; revised edition published March 1985, 276 pp.
- Vol. 32 – Fishery statistics for 1982, Published December 1984, 284 pp.
- Vol. 33 – Fishery statistics for 1983, Published December 1985, 280 pp.
- Vol. 34 – Fishery statistics for 1984, Published December 1986, 304 pp.
- Vol. 35 – Fishery statistics for 1985, Published December 1987, 322 pp.
- Vol. 36 – Fishery statistics for 1986, Published October 1989, 304 pp.
- Vol. 37 – Fishery statistics for 1987, Published April 1990, 295 pp.
- Vol. 38 – Fishery statistics for 1988, Published February 1991, 307 pp.
- Vol. 39 – Fishery statistics for 1989, Published February 1993, 300 pp.
- Vol. 40 – Fishery statistics for 1990, Published February 1994, 309 pp.
- Vol. 41 – Fishery statistics for 1991, Published February 1995, 318 pp.
- Statistical Bulletin Supplementary Issue, 1960–90, (statistics) Published April 1995, 156 pp.
- Vol. 42 – Fishery statistics for 1992, Published October 1995, 310 pp.
- Vol. 43 – Fishery statistics for 1993, Published December 1997, 329 pp.
- Vol. 44 – Fishery statistics for 1994, Published December 2000, 201 pp.
- Vol. 45 – Fishery statistics for 1995, Published October 2001, 207 pp.
- Vol. 46 – Fishery statistics for 1996, Published November 2001, 214 pp.
- Vol. 47 – Fishery statistics for 1997, Published November 2001, 216 pp.
- Vol. 48 – Fishery statistics for 1998, Published November 2001, 210 pp.
- Vol. 49 – Fishery statistics for 1999, Published January 2002, 210 pp.

Inventory of Sampling Data

This publication replaced *ICNAF Inventory of Sampling Data 1967–1978* which was completed in 1986.

- Inventory of Sampling Data 1979–1984, Published April 1989, 250 pp.
- Inventory of Sampling Data 1985–1989, Published March 1993, 265 pp.
- Inventory of Sampling Data 1990–1994, Published October 1999, 287 pp.
- Inventory of Sampling Data 1995–1999, Published November 2002, 142 pp.

NAFO Index of Meeting Documents

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

- 1979–84 – Index of Meeting Documents, Published March 1985, 146 pp.
- 1985–89 – Index of Meeting Documents, Published December 1990, 116 pp.

NAFO Index of Meeting Documents (Continued)

- 1990–94 – Index of Meeting Documents, Published November 1995, 139 pp.
1995–99 – Index of Meeting Documents, Published December 2000, 141 pp.
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NAFO Scientific Council Studies

Previous Issues

- No. 1. Miscellaneous Selected Papers (101 pages, published March 1981)
- No. 2. Manual on Groundfish Surveys in the Northwest Atlantic. W. G. Doubleday, Editor (55 pages, published December 1981)
- No. 3. Miscellaneous Selected Papers (82 pages, published April 1982)
- No. 4. Special Session on Remote Sensing, September 1981 (98 pages, published September 1982)
- No. 5. Symposium on Environmental Conditions in the Northwest Atlantic During 1970–79, September 1981 (113 pages, published December 1982)
- No. 6. Miscellaneous Selected Papers (103 pages, published December 1983)
- No. 7. Miscellaneous Selected Papers (97 pages, published August 1984)
- No. 8. Miscellaneous Selected Papers (95 pages, published April 1985)
- No. 9. Special Session on Squids, September 1984 (179 pages, published November 1985)
- No. 10. Miscellaneous Selected Papers (112 pages, published August 1986)
- No. 11. Miscellaneous Selected Papers (128 pages, published March 1987)
- No. 12. Miscellaneous Selected Papers (90 pages, published March 1988)
- No. 13. Miscellaneous Selected Papers (82 pages, published November 1989)
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- No. 15. Miscellaneous Selected Papers (68 pages, published May 1991)
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- No. 20. Miscellaneous Selected Papers (113 pages, published February 1994)
- No. 21. Collection of Papers Related to Northern Cod and Seals in NAFO Divisions 2J and 3KL, Papers from June 1993 (165 pages, published December 1994)
- No. 22. Miscellaneous Selected Papers (95 pages, published May 1995)
- No. 23. Miscellaneous Selected Papers (95 pages, published September 1995)
- No. 24. Symposium on Impact of Anomalous Oceanographic Conditions at the Beginning of the 1990s in the Northwest Atlantic on the Distribution and Behaviour of Marine Life (155 pages, published January 1996)
- No. 25. Flemish Cap Selected Environmental and Other Papers (91 pages, published July 1996)
- No. 26. Selected papers on Harp and Hooded Seals (129 pages, published December 1996)
- No. 27. Miscellaneous Selected Papers (81 pages, published December 1996)
- No. 28. Assessment of Groundfish Stocks Based on Bottom Trawl Survey Results (105 pages, published December 1996)
- No. 29. Selected Studies Related to Assessment of Cod in NAFO Divisions 2J+3KL (125 pages, published May 1997)
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- No. 31. Miscellaneous Papers (165 pages, published December 1998)
- No. 32. Miscellaneous Papers (133 pages, published April 1999)
- No. 33. Miscellaneous Papers (135 pages, published May 2000)
- No. 34. Miscellaneous Papers (91 pages, published October 2001)

