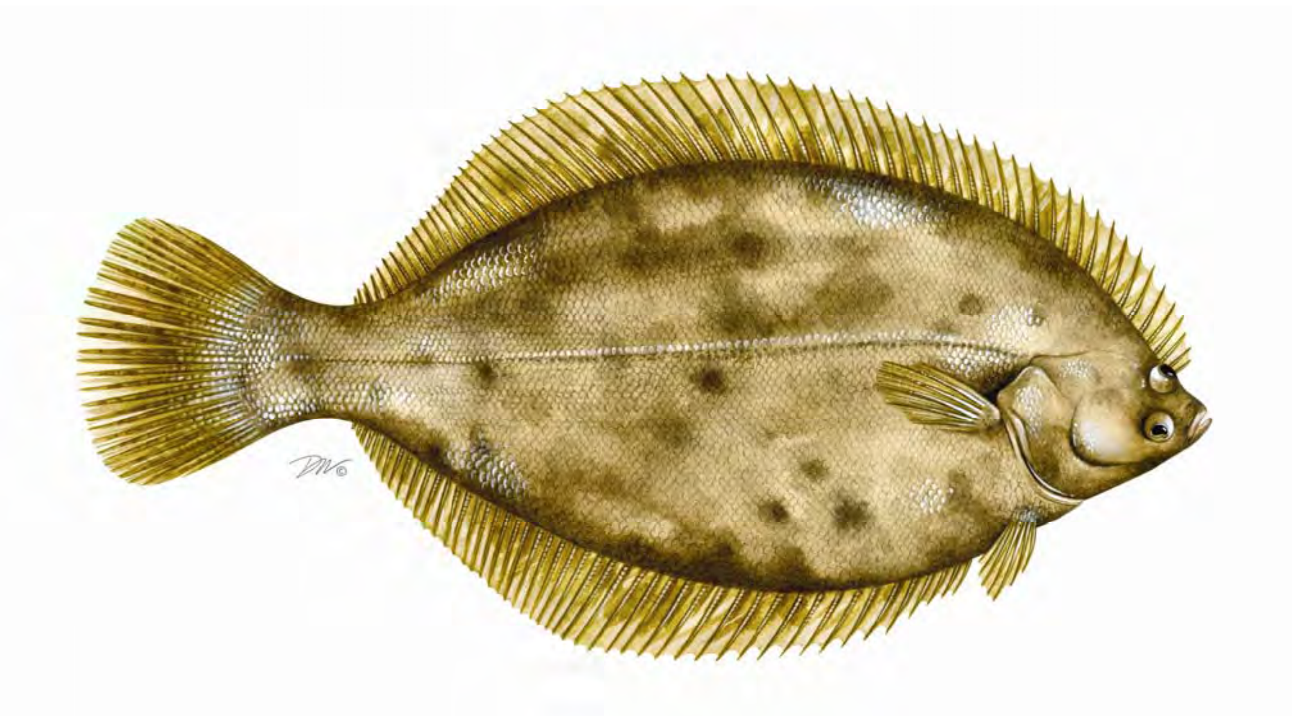
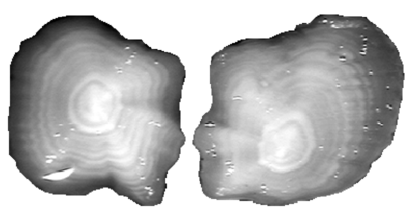
Atlantic States Marine Fisheries Commission

Healthy, self-sustaining populations for all Atlantic coast fish species or successful restoration well in progress by the year 2015





Proceedings from the Winter Flounder Ageing Workshop

February 2012

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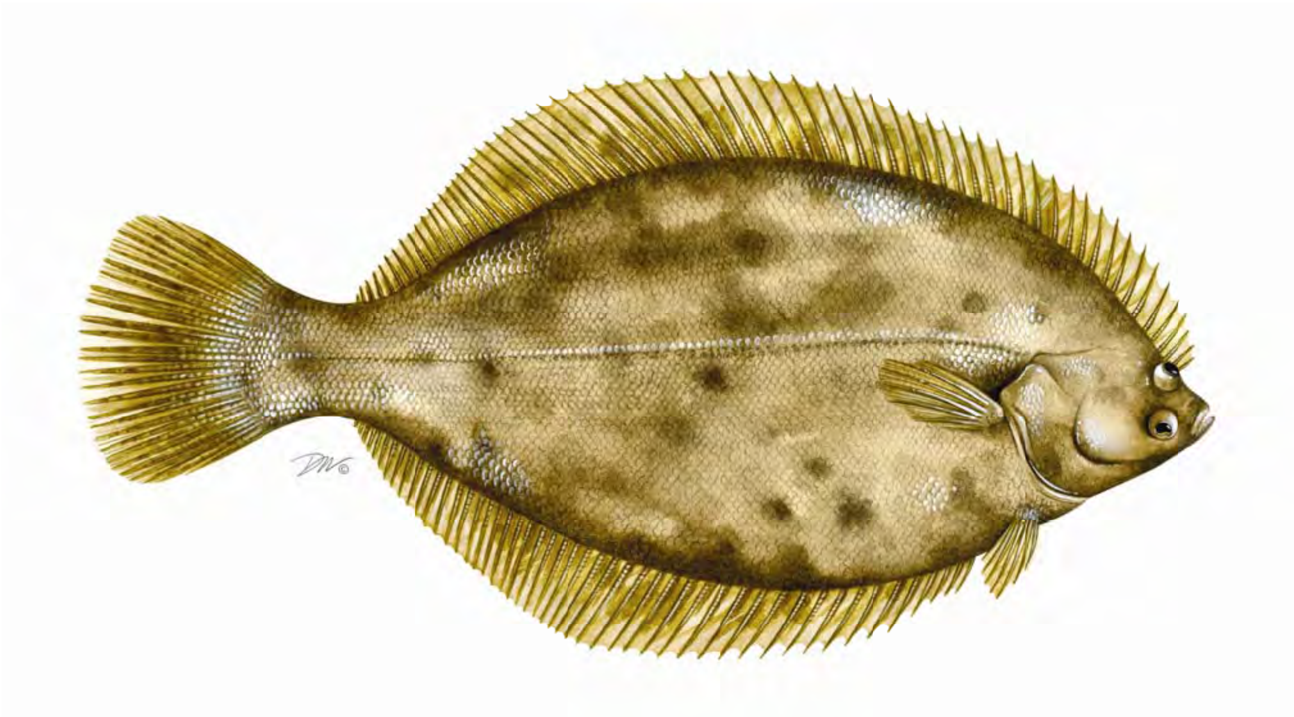

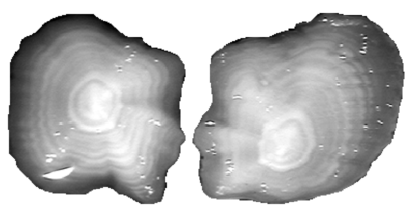

Held at the National Marine Fisheries Service
Northeast Fisheries Science Center
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Introduction

The Atlantic States Marine Fisheries Commission's Winter Flounder Technical Committee conducted a winter flounder otolith aging comparison study in 1998, to measure interpretative consistency among four readers (Appendix I). Bias measured by this study related to systematic differences detected among comparisons between readers, but did not address accuracy relative to true age.

Overall, the results suggested that interpretations among the four readers were inconsistent; especially on age 4^+ fish, which are considered fully recruited. Inconsistency is noted in low percent agreement, low precision, and bias. One reader's interpretations were very inconsistent with the other three readers, due primarily to interpretation of the first annulus and subsequent annuli. Bias was evident even in the best two-reader comparison. Inconsistency (especially bias) increased with age, suggesting either heterogeneous criterion among readers for discriminating spawning checks from annuli and/or interpretation of the edge type (hyaline or opaque) among these readers.

The inconsistency identified in the study may have been somewhat inflated compared with inconsistency occurring within laboratories. Ages were assigned to whole otoliths only, without the option of sectioning difficult otoliths or the use of scales (the primary structure typically used by one reader). In addition, the four readers may have perceived pressure to provide age interpretations on structures whose condition normally would have precluded age assignment. Finally, readers were asked to interpret structures from fish taken outside their geographical area or taken during a slightly different time-period. Interpreting ages of fish with unfamiliar growth patterns or blindness to the collection date may have inflated inconsistency.

The results suggested the need for a workshop to develop consensus criteria for defining annuli and interpreting edge type for winter flounder. The ASMFC sponsored a Winter Flounder Ageing Workshop on August 22-23, 2001, held at the National Marine Fisheries Service's Northeast Fisheries Science Center in Woods Hole, Massachusetts (Appendices II, III, and IV). The following terms of reference were adopted by the workshop:

- To evaluate the various structures and methods to age winter flounder.
- To develop standardized protocols for ageing, training, testing, and evaluating consistency.
- To produce a Workshop Proceedings document.

General Biology of the Species[1]

The winter flounder, *Pseudopleuronectes americanus*, is a small-mouthed, right-sided flatfish distributed in coastal waters from Labrador to Georgia and offshore on the Georges Bank. Winter flounder grow to a maximum length of about 67 cm (Collette and MacPhee 2001) and live to approximately 15 years of age (Kennedy and Steele 1971; Howe and Coates 1975; Fields 1988; J. Burnett, pers. comm.). They feed on benthic invertebrates, primarily polychaete worms. For coastal populations, spawning occurs in late winter-early spring in brackish waters of estuaries and salt ponds, followed by movement of adult fish to deeper water during summer and fall. Tagging studies indicate that winter flounder generally return to the same spawning location, suggesting the existence of several discrete local groups. For management purposes, three groups of winter flounder in U.S. waters are recognized and assessed: Gulf of Maine, Southern New England-Middle Atlantic, and Georges Bank. Statistical areas used in stock definitions for winter flounder in United States waters are found in Appendix V.

The Southern New England/ Mid-Atlantic stock complex is distributed on the shelf from south and east of Cape Cod to New Jersey, shoreward of the Great South Channel. For females, the age of 50% maturity is 3.0 years (O'Brien et. al. 1993). The assessment uses ages 1-6 with a 7+ plus group (NEFSC 1999). In recent years, fish are partially recruited beginning at age 2 and are fully recruited at age 4. The fishery is mostly commercial with otter trawls accounting for 95% of the commercial landings. In recent years, recreational catch accounted for approximately 20% of the total landings.

The Georges Bank winter flounder stock is distributed on the shallower portions and has higher growth rates than the inshore stocks. For females, the age of 50% maturity is 1.83 years (NEFSC 1999). In the most recent years, Georges Bank winter flounder begin to recruit to the fishery at age 2 and are fully recruited at age 4. The assessment uses ages 1-6 with a 7+ age group. Recreational catches are insignificant and 95% of the commercial landings are taken by otter trawl.

The Gulf of Maine winter flounder stock is distributed in shallow coastal waters from Cape Cod Bay to Nova Scotia (NEFSC 1995). Winter flounder in Massachusetts' waters north of Cape Cod grow slower and mature later than those in Massachusetts' waters south and east of Cape Cod (Witherell and Burnett 1993). For females, age of 50% maturity was 3.3 years. The Gulf of Maine winter flounder assessment is index-based with estimates of mortality derived from survey catch curve analysis using ages 4-7 (Cadrin et al. 1996). Both commercial and recreational fisheries exploit the stock. Recreational landings were a significant proportion of total landings in the early 1980's, but as a proportion of total landings have declined markedly in recent years. Commercial landings are harvested with otter trawls and gillnets.

As with most flatfish, winter flounder exhibit dimorphic growth and maturation rates: males generally mature earlier but grow to a smaller maximum size than females. Regionally, growth rates are highest for Georges Bank individuals, while coastal fish exhibit a gradient of decreasing growth rates from south to north. Recent published studies of winter flounder age and growth include Lux (1973(a) (b)) for the Georges Bank and Witherell and Burnett (1993) for Massachusetts' waters. O'Brien et al. (1993) report maturation rates for winter flounder on Georges Bank and fish north and

[1] Please refer to the SAW 52 stock assessment report (NEFSC 2011) for the most up to date life history and stock status information.

south of Cape Cod. Haas and Recksiek (1995) conducted an age verification study of winter flounder in Narragansett Bay, summarized in Appendix VI.

Winter Flounder Ageing Workshop Proceedings

Standardization of Age Reading

Please see Appendix VII for a glossary of terms (from Penttila and Dery 1988) used in the text.

Scales

Workshop participants agreed to use the following description of age reading for scales, taken from Penttila and Dery (1988) (Figure 1):

> By convention, a 1 January birthdate is used. Annular zones on winter flounder scales appear as changes in the circuli pattern. Zones of fast and slow growth are reflected by wide and narrow spacing, respectively, of circuli, made up of individual platelets on the sculptured upper surface of the scale.
>
> On winter flounder scales and otoliths, the first winter zone representative of the first annulus is well defined for slow growing fish but not for fast growing fish. The scale winter zone appears on the edge approximately coincident with the hyaline edge on otoliths. Studies have demonstrated close agreement between scale and otolith readings from the same fish through age 4.
>
> The first annulus on a scale is identified by a dense mass of winter growth (closely spaced circuli) near the focus; the end of the annulus is considered to be the outermost of these circuli (see Figure 1 in Penttila and Dery 1988). Sometimes pigmentation on the scale will cover the first annulus almost completely. The first annulus on many scales is barely discernible and is usually estimated by slight changes in formation of the circuli. For all succeeding years, spring and summer growth are characterized by widely spaced circuli (rapid length accretion) and fall and winter growth by closely spaced circuli (slow length accretion). The outer edge of the zone of closely spaced circuli on the scale are considered to be checks and may be ignored in assigning age.
>
> On scales from older fish the identity of checks is more obvious with the more strongly formed annuli (see Figures 3 and 4 in Penttila and Dery 1988). After formation of the third annulus, irregular spacing of annuli (see Figure 5 in Penttila and Dery 1988) may complicate age interpretation. The growth increment between the second and third annuli is generally wide, with decreasing growth increments between later annuli (see Figures 6 and 7 in Penttila and Dery 1988).
>
> Contrast between winter and summer zones tends to deteriorate towards the outer edge of scales of older winter flounder. After the fourth winter zone, summer growth appears to merge with the slow winter growth and the narrow growth increments may make interpretation difficult (see Figure 8 in Penttila and Dery 1988).

Interpreting Scales

Scale samples are taken from the caudal peduncle. Information from multiple scales may be combined to build a complete age interpretation when clarity of growth patterns from a single scale is insufficient (Figure 2). Annuli can be followed into lateral fields while spawning checks do not. Breaks or curvature in radii often occur at the annulus. Regenerated scales are useful to prompt readers to look for possible false annuli formation (from a stressful event which caused scale loss) in the same position that growth resumed in regenerated scales.

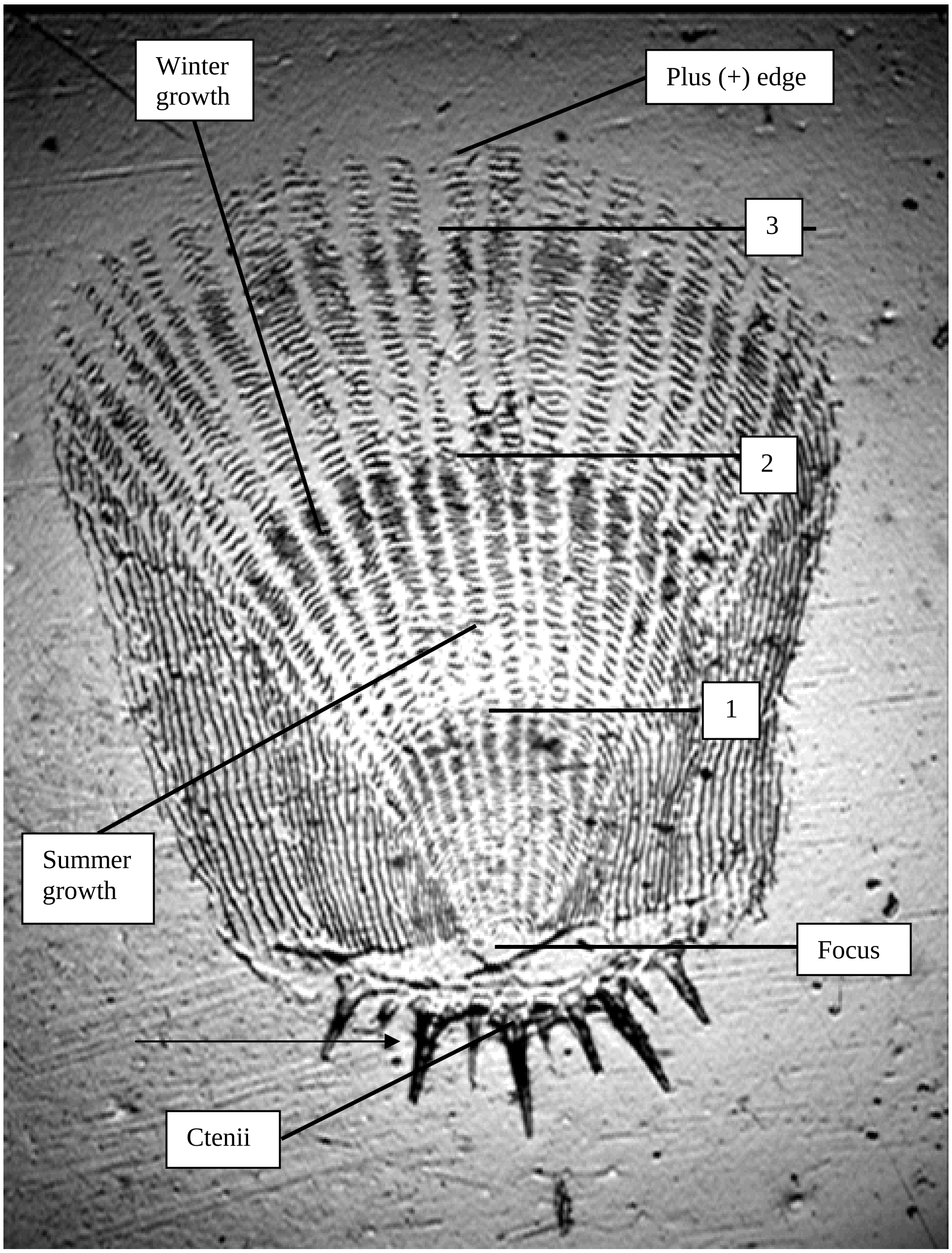

Figure 1: Winter flounder scale from a 24 cm female collected in the Gulf of Maine, July 1993. Aged 3+. Illustrates the ease of ageing younger winter flounder with scales.

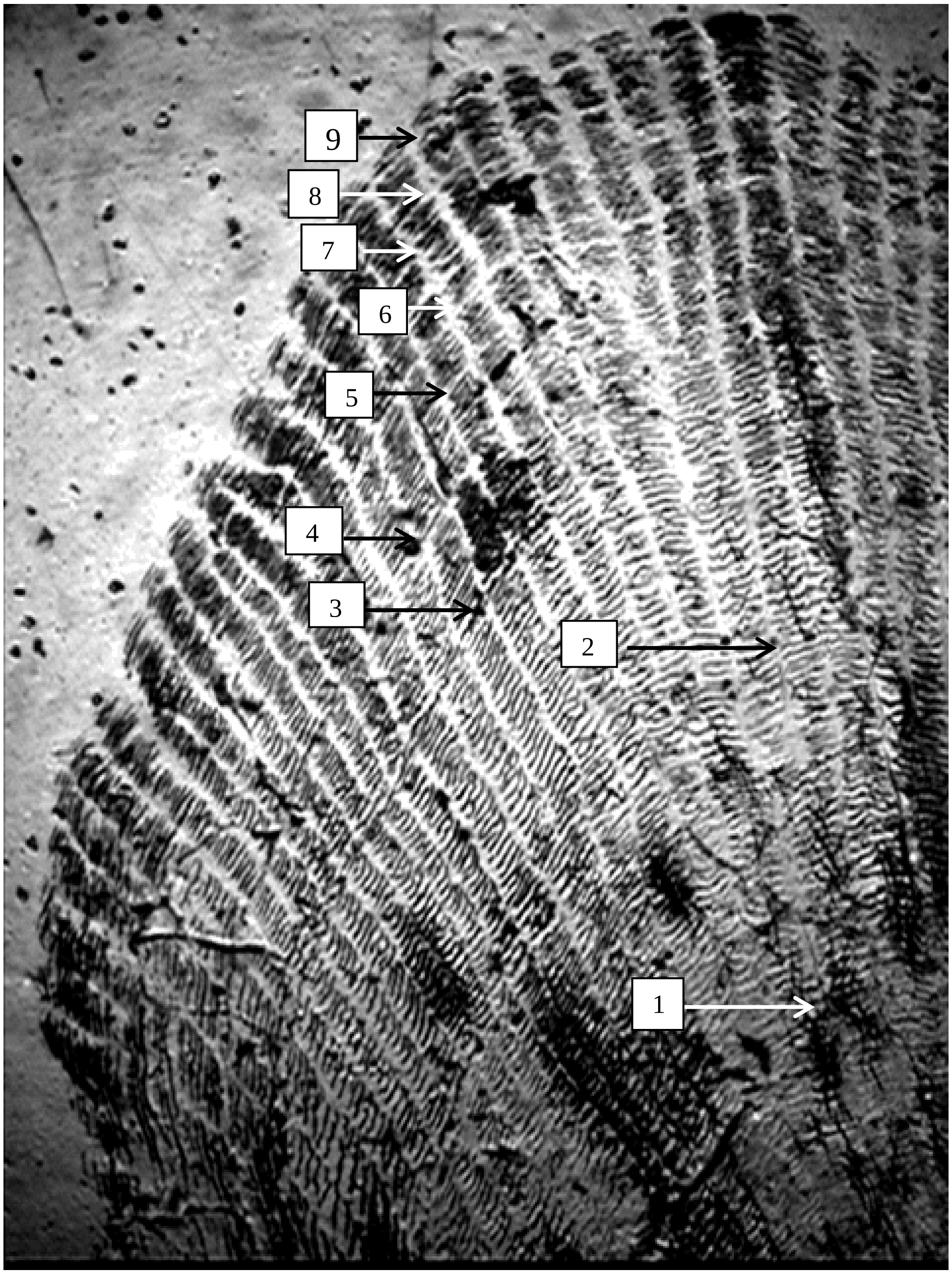

Figure 2: Scale collected from a 49 cm female from the Gulf of Maine, July 1993. Aged 9+. Detail of scale edge illustrates the difficulty of ageing larger fish with scales due to the crowding of annuli.

Otoliths

Larval winter flounder undergo a metamorphosis in which the left eye migrates from the left side of the fish's head to the right side of the head prior to settlement. Flounder then assume a benthic lifestyle, lying on their eyeless side. As a result, we refer to the "eye side" (right side) and the "blind side" (left side) of winter flounder. Winter flounder are right-eyed flounder, defined by having their eyes are on the right side of their bodies and their left side is the blind side. The otoliths of the flounder do not change position during the migration of the eye and are separated by the mid-sagittal plane. The two otoliths lie along a lateral axis which is perpendicular to both the anterior-posterior axis and the dorsal-ventral axis (Figure 3). The unusual orientation of flatfish of lying on one side compared to roundfish can lead to common but inaccurate usage of anatomical descriptions in the field (e.g., eye side=up=dorsal, blind side=bottom=ventral). In this report, we refer to the right otolith as the eye side otolith and the left otolith as the blind side otolith.

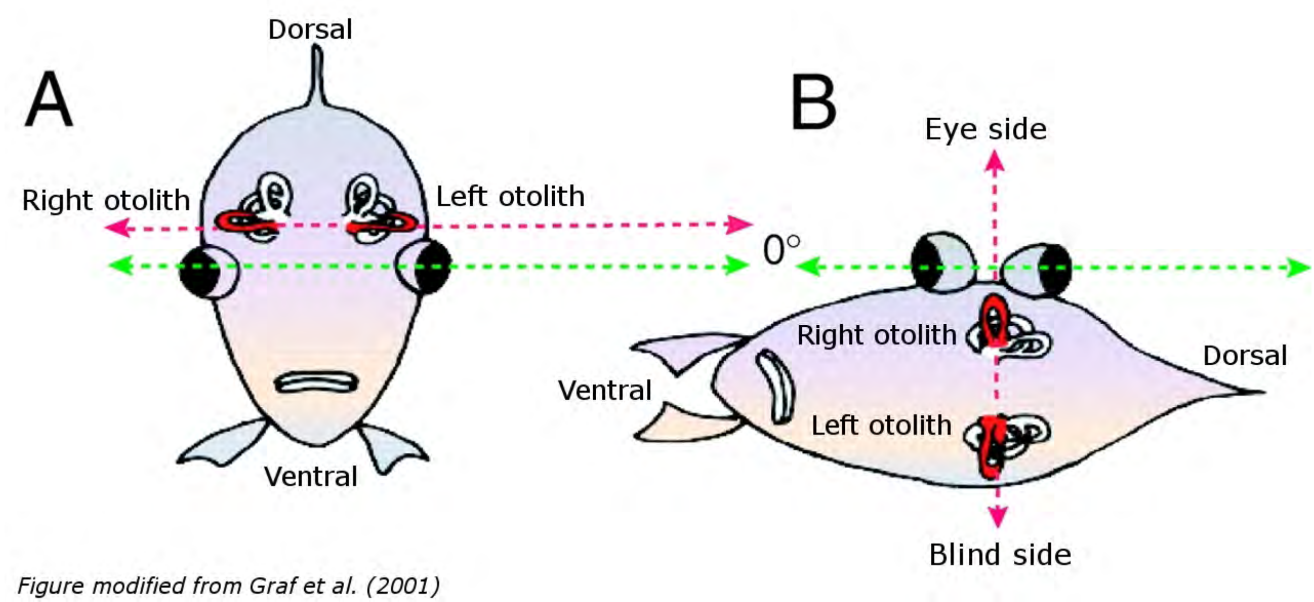

Figure 3: Schematic to illustrate eye migration and location of otoliths in right-handed flatfish for larval (A) and post-metamorphic (B) animals. Green axis is eye axis. Red axis is lateral axis.

Interpreting the center of whole otoliths may be confusing (settling check, etc.). In sectioned otoliths, apparent splits and checks tend to coalesce into more defined annuli near the sulcus (Figure 4). Splitting patterns may be cause for ignoring marks which otherwise meet annulus criteria. Splits often repeat themselves serially throughout the otolith. Split zones should be lumped if consistent with expected growth patterns.

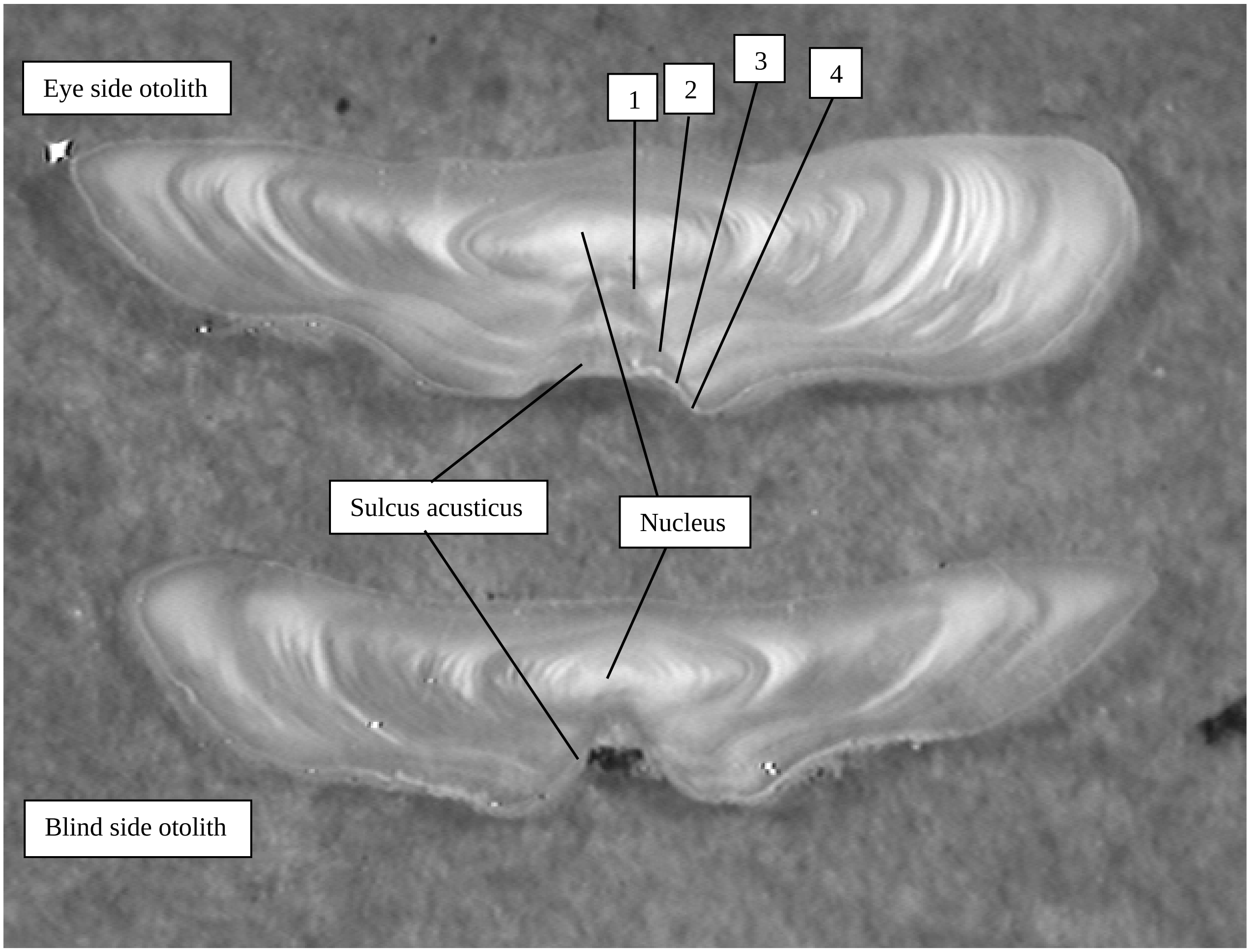

Figure 4: Otolith thin sections from a 27 cm male winter flounder collected in April 2000 during the NEFSC spring bottom trawl survey in the Gulf of Maine (Scotian Shelf). Aged as 4. Sections are from the eye side (right otolith) and blind side (left otolith) otoliths.

Good quality otoliths are useful to develop a search image for interpreting unusual patterns (splits, settling checks, etc.). Ideally, annuli should appear as continuous bands throughout the otolith (Figure 5 and Figure 6). Checks are generally visible in only 30% of the viewing field used for ageing whereas annuli cover nearly 95%. For whole otoliths, adherence to this protocol may not be rigid, but it should be used in most sectioned otoliths. Annuli generally are continuous through younger ages. In older fish annuli become crowded and may be difficult to follow completely around the whole otolith (Figure 7). Because of this crowding at the edge, sectioning the otoliths of larger fish (>40-50 cm, or where only the thicker eye side (right) otolith has been collected) is often necessary. Annuli can be easier to distinguish at the edges of sectioned otoliths (compare Figure 7 and Figure 8).

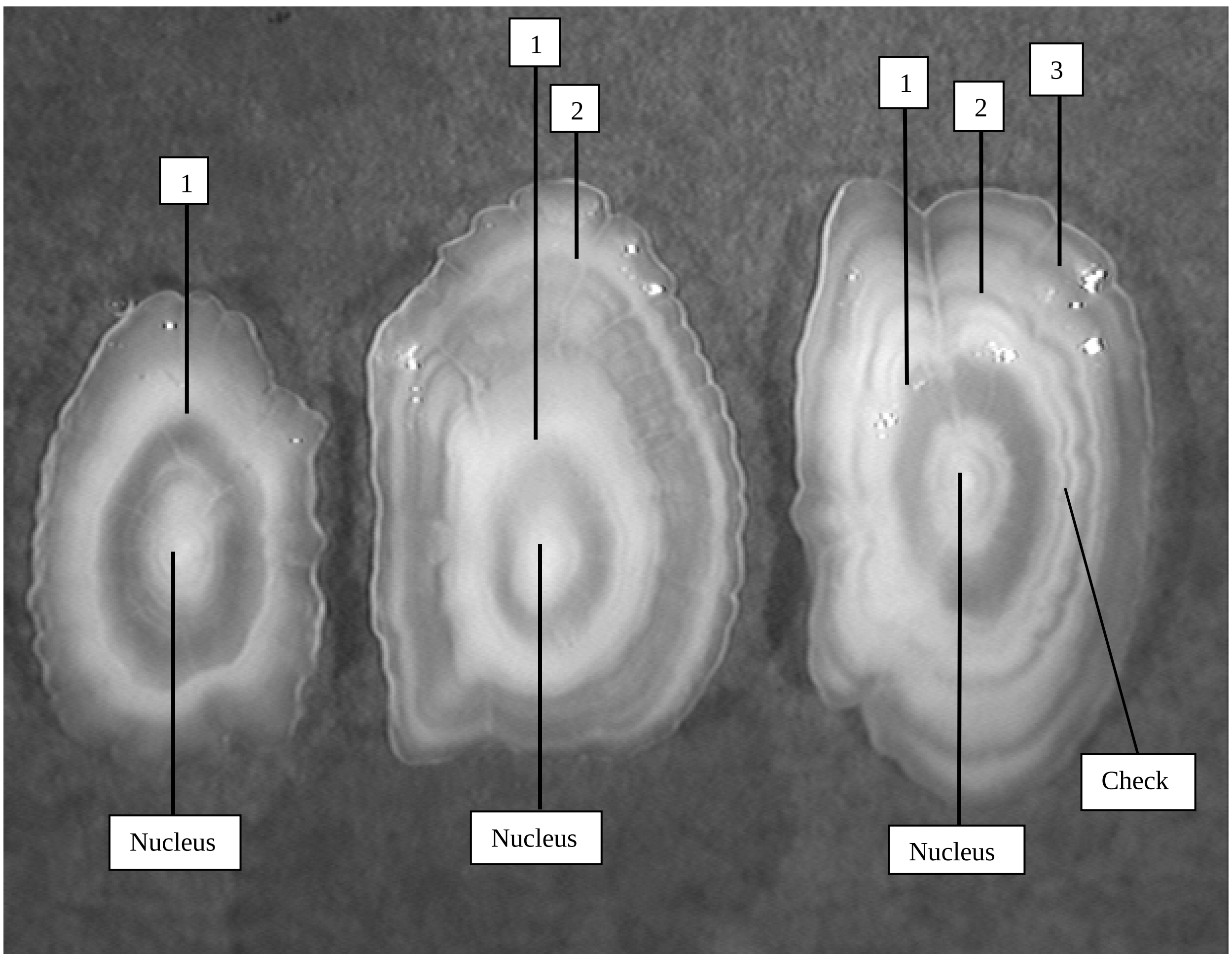

Figure 5: Winter flounder otoliths collected during the NEFSC 2000 autumn bottom trawl survey in the Southern New England region. Left to right: a 23 cm female aged as 1+, a 30 cm female aged as 2+, and a 35 cm female aged as 3+.

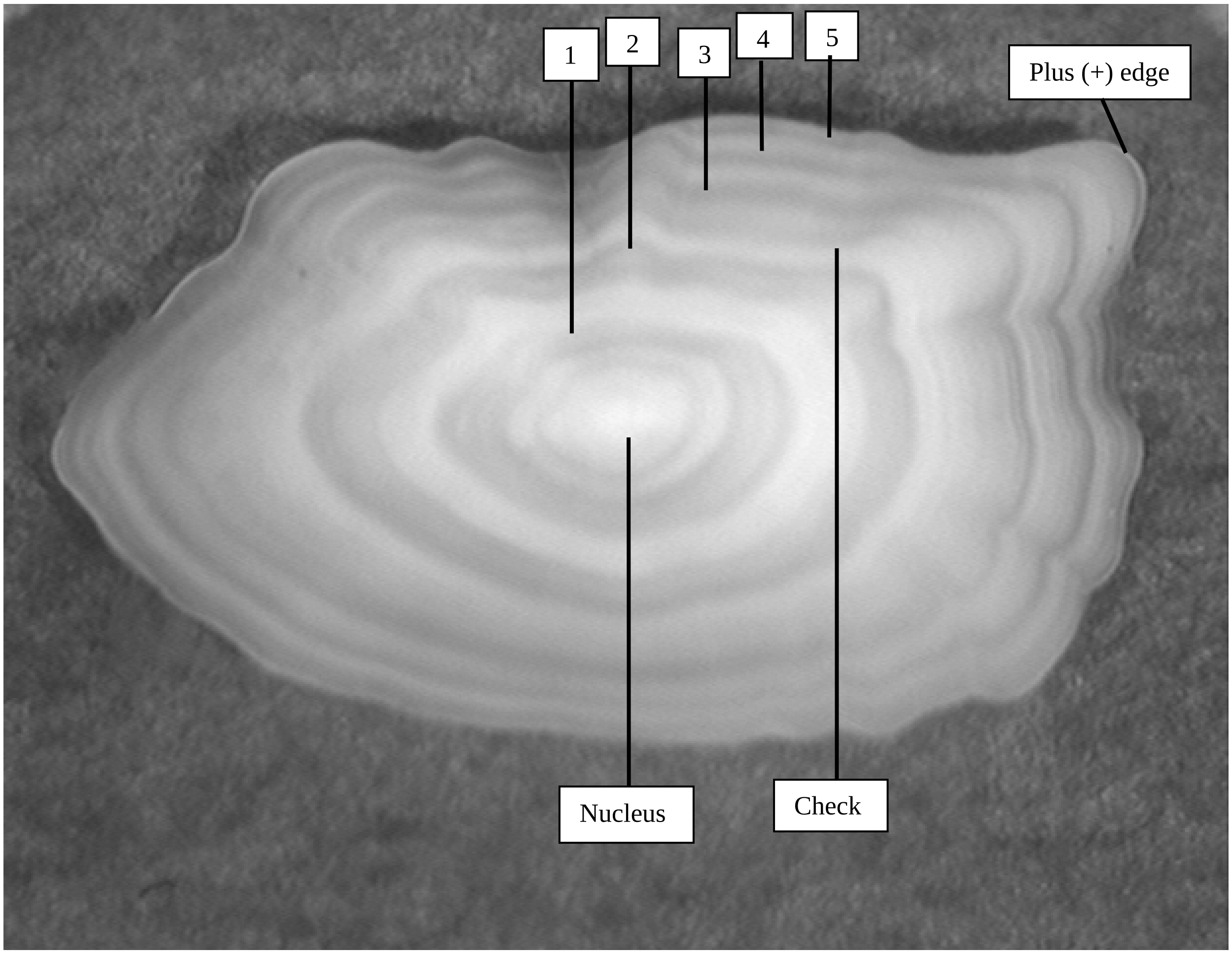

Figure 6: Otolith collected from a 29 cm male winter flounder during the NEFSC 1993 summer bottom trawl survey in the Gulf of Maine. Aged as 5+. Note the opaque edge visible beyond the fifth annulus and the strong check mark between the 2nd and 3rd annuli.

Growth patterns should be considered when determining whether a mark is an annulus or a check. Unique regional growth patterns may add to complexity in interpretation. Every estuary/spawning zone may have unique patterns, particularly in the first year of growth. Inshore winter flounder experience a wider range of environmental factors and as a result their otoliths may be more difficult to interpret than those from offshore fish. High magnification may result in interpreting insignificant marks as annuli and confound interpretation by not allowing a look at the entire pattern on the otolith. Dissecting scopes at 2 to 6 X power are commonly used for whole otoliths while 12 to 25 X power magnification is used for otolith sections. The anterior and dorsal tips of the otolith exhibit earliest edge material deposition and readers should examine this area for edge definition.

Canada (DFO) uses the following criteria to interpret edge:

Zone width	Narrow	Wide
Edge type	Hyaline/opaque	Hyaline/opaque

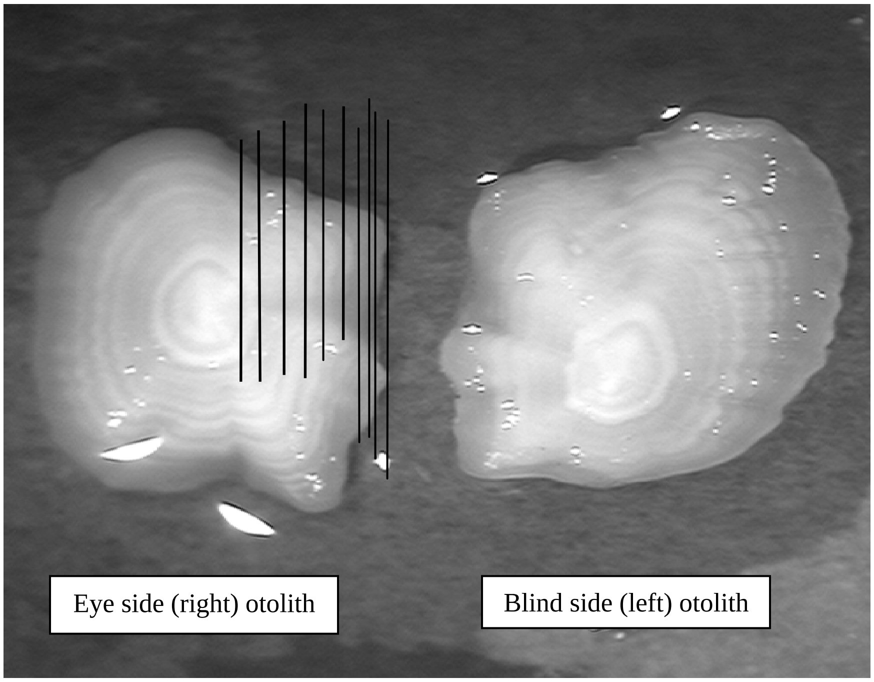

Figure 7: Eye side and blind side otoliths collected from the same 49 cm female described in Figure 2. Aged at 10+. Annuli are crowded on the otolith edges and are poorly seen; these otoliths would be selected for thin sectioning.

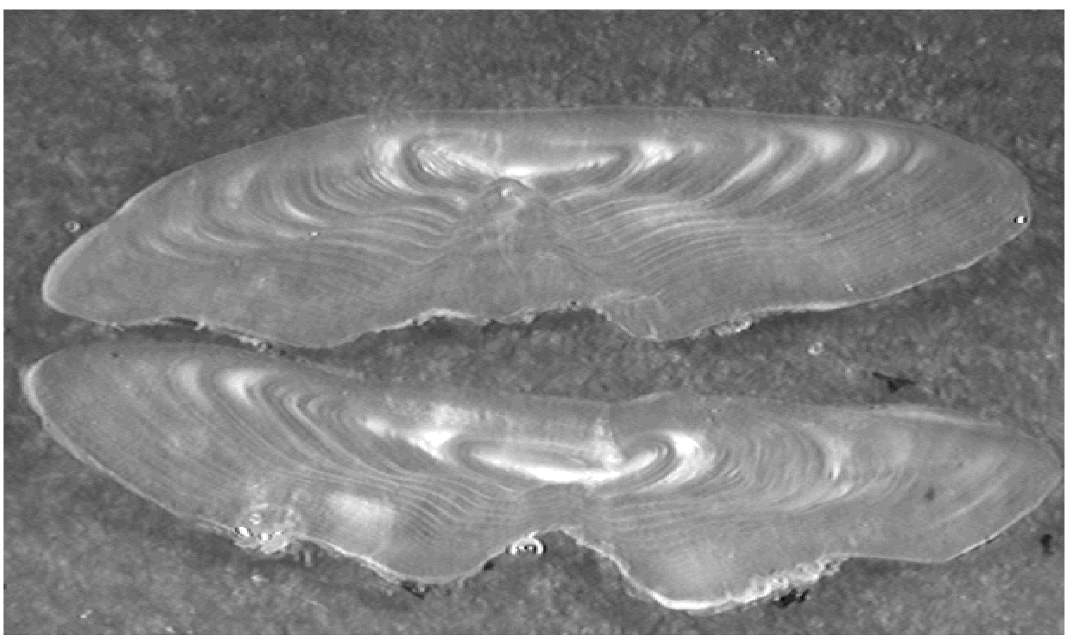

Figure 8: Sectioned otoliths from the same 49 cm female described in Figure 2 and Figure 6. Aged at 13+. Not captioned due to quality of the scanned image.

Season of capture is important in age determination. Workshop participants discussed the "50% rule", in which a year is added to the number of annuli present if the last opaque increment is greater than 50% of the previous opaque zone. However, the group did not recommend a standardized method of edge definition beyond determining hyaline versus opaque.

Age Reading Comparisons (scales vs otoliths)

In 1993, the NEFSC conducted an age reading comparison of scales vs otoliths. The methodology and results from that study are summarized as follows:

> Scales and otoliths were collected from 320 winter flounder during the NEFSC Gulf of Maine summer bottom trawl survey (R/V Delaware II Cruise No. 93-08, July 20-August 4). Catches of winter flounder occurred primarily in coastal waters (less than 30 fathoms) ranging from Grand Manan Island to Cape Cod Bay.
>
> Scales were impressed onto laminated plastic as described by Penttila et al. (1988) and read at 43X magnification using a Dawson microprojector. Age determinations were made according to criteria provided by Fields (1988). Whole otoliths were viewed at magnifications of 12-25X through a binocular microscope using reflected light; droplets of commercial dishwashing detergent were applied to enhance the clarity of otolith zones. Age determinations were based upon the number of completed hyaline zones adjusted for timing of annulus formation, similar to the procedures of Haas and Recksiek (1995) for thin-sectioned otoliths. Scale impressions and whole otoliths from each sample were read independently without knowledge of the age determination provided by the alternate structure.
>
> **Results:** Age determinations were obtained from both scales and whole otoliths from 279 winter flounder. Thirty-seven samples contained no otoliths and one sample contained no scales. Additionally, one pair of otoliths could not be read due to crystallization and two scale samples were unreadable - one because all scales were regenerated and one due to the absence of defined annuli.
>
> Age determinations ranged from age 1 to a maximum of age 8 (scales) and age 10 (whole otoliths), with 86 % of the sample comprised of ages 2-4. The same age was determined from both structures for 95% of the total sample. Of the 15 total disagreements, scale age was one year greater than the otolith age in seven instances, scale age was one year less than the otolith age in seven instances, and scale age was two years less than the otolith age in one instance (Table 1).

The disagreements between scale and otolith ages can be classified into three types. In four samples, the otolith provided a better basis for observing and interpreting annuli beyond age 6 (three fish aged 8 using otoliths and 7 by scales, and one fish aged 10 using otoliths and 8 by scales). Opaque and hyaline zones were distinct throughout the otoliths to the full age, while the scale margins were crowded with circuli, making annulus determination difficult. This difficulty in viewing scale margins also contributed to the apparent over-ageing by one year in two other samples, where poorly defined scale events close to the edge were counted as an extra annulus relative to the ages determined from unambiguous otolith zones. The third type of disagreement (a total of nine, involving both the under-ageing and over-ageing by one year of fish ages 2-5) was related to the discrepancy between the timing of annulus formation within the two age structures. Annuli in scales appear to form very consistently during March-April, while completion of otolith hyaline zones appears highly variable between individuals and occurs over a protracted period from October to May [this phenomenon was also observed by Haas and Recksiek (1995) for winter flounder in Narragansett Bay]. Age determination disagreements for the nine fish in question were resolved after additional careful examination of the otoliths was made relative to the possibility of ‘early’ or ‘late’ annulus formation. This exercise points out that caution must be used in interpreting otolith edge type**.**

Table 1: Results of a 1993 NEFSC study comparing age determinations obtained from scales (rows) and whole otoliths (columns) from 279 individual winter flounder collected in the Gulf of Maine. Bold numbers indicate number of age determinations in agreement.

		Otolith Age									
		1	2	3	4	5	6	7	8	9	10
Scale Age	1	**4**									
	2		**82**	2							
	3		3	**98**							
	4			1	**51**	2					
	5				1	**19**					
	6						**6**				
	7						1	**4**	3		
	8							1			1
	9										
	10										

Workshop Discussion

Workshop participants expressed discomfort with relying solely on whole otoliths to age winter flounder, as whole otoliths vary in clarity. Age and length do not always determine otolith reading quality, though the whole otoliths from older fish are most likely to be difficult to interpret. Based on the limited sample examined during the workshop, precision appeared to be low on older fish aged with whole otoliths. The asymmetric otolith (blind side) is better for ageing whole and the symmetric otolith (eye side) is preferred for sectioning. This implies the importance of collecting both otoliths when sampling. Confidence in assigning a single age on large/old fish may be low, however, and since relatively fewer old fish are collected, greater effort to determine an age is warranted.

Scales of young fish appear to be easily interpreted, but interpreting scales from older, larger fish is often limited by annuli crowding at the edge. Comparison studies reported by Ruth Haas-Castro at this meeting and others show good agreement between otoliths and scales younger than age 5. Evidence exists that many species have been underaged when scales were relied upon for ageing older fish (Beamish and McFarlane 1987). Whole otoliths also may have resulted in underaging of older samples (Walsh and Burnett 2002). This phenomenon should be examined for winter flounder, especially ages 5+.

The trade-off between precision associated with using a particular structure and methodology, and availability of resources (time, personnel) should be considered in sampling design. A hybrid of methods depending on age may be useful (e.g., scales/whole otoliths for young fish and whole/sectioned otoliths for older fish). In some cases, preferred structures may not be available, resulting in lower precision in ageing. Reporting confidence by age may be useful for end-users of age data. For example, if scales are not reliable past age X, then the plus group in a catch-at-age matrix developed with scales should not exceed age X^+.

A discussion on the use of length information when determining age revealed some procedural differences among workshop participants. Several readers routinely utilize lengths when ageing and both request samples by length prior to ageing. They believe that knowing length *a priori* assists with interpreting age patterns. Canadian procedures note that ages are determined with no length information to avoid bias. One reader noted that experienced age readers are able to estimate the size of the fish based on the size of the age structure anyway, and that differing growth patterns limit the utility of knowing the length. Workshop participants made no recommendation regarding the use of length information when determining age.

Workshop Comparison Results

Overall, readers were generally able to reach consensus age on samples examined at this meeting. The ability to reach consensus combined with good agreement between scales and otoliths at younger ages suggested that confidence in the historical ageing time series is warranted. However, reaching consensus required substantive discussion of observed patterns in age structures. The alternative interpretations of marks, made outside of a group setting, may have resulted in bias and the high CV's reported by Correia and King's (2001) study (attached as Appendix I).

Several methods to examine historical series were proposed, including comparing trends in mean weight at age, mean length at age, and maturity at age. A second method for examining the historical time series is to re-examine a subsample of historical ages after calibration with a reference collection. The latter method is more time-consuming but allows quantification of consistency. Workshop attendees concluded that the use of sectioned otoliths could have reduced some of the disagreement in the 4-way comparison study as well as substantially improving the agreement on samples examined at the workshop.

Workshop participants concluded that age determinations for winter flounder (up to age 5) could be made just as reliably from whole otoliths as from scales. Moreover, whole otoliths appear to be superior to scales for ageing larger, older fish. The confounding effect of the timing of otolith annulus formation (described above) can be easily recognized, involves only a small proportion of fish, and should not be construed as a negative factor associated with otolith usage.

Workshop Recommendations

1. Establish a reference collection by stock area (highest priority/urgent)

> **a) Establish a working group to determine best mechanism to create and establish reference collections for both scales and otoliths (whole and sectioned). Agencies should collect and prepare age samples to build the reference collections.**
>
> **b) In the near-term, ageing should continue without reference collections, but establishing the reference collections is high priority.**

Reference collections will allow the precision and bias of age data to be quantified, allow consistency to be measured over time, and would be invaluable training tools. Utilization of reference collections is the keystone of a quality control program. Rigorous implementation of reference collections prevents interpretations from "drifting" through time and helps ensure consistency of age interpretations among readers. Development and use of reference collections allows quantification of precision and bias for ageing results. A reference collection of "known" ages allows quantification of accuracy of age interpretations and decreases the need for secondary age readers. Reference collections are useful for training purposes and for maintaining consistency within and among readers. An additional benefit is that end users of age data could make informed decisions based on the diagnostic results (e.g. choice of assessment model, age at which to lump into plus group, etc.). Consensus age samples from this study (by area) may be a first start. It was suggested that reference collections be set up with guidance from the Canada-United States Yellowtail Flounder Age Reading Workshop (Walsh and Burnett 2002).

The samples should be representative of all data types (surveys, commercial, recreational), gear types (trawls, gill nets, hook and line, etc.), and population structures (sex, length, age, and area (within each stock region)). Samples from several collection years should be represented. Reference collections should consist of samples of known age. When known age samples are not available, a collection of samples aged by experts who reached consensus will suffice.

The group discussed posting digital pictures of the reference collection on the Internet, but the general conclusion was that hard part samples should be exchanged so each laboratory can assign age. Age readers can discuss any samples where disagreement occurs, but care must be exercised to prevent unequal influence from individual age reader(s) in this step. Collections should be of sufficient size so readers cannot memorize the samples. Collections should be dynamic with new samples added routinely from recently read, consensus-aged material.

A subsample of 200 structures selected from past years completed and accepted collection should be read prior to commencing production ageing. If bias and CV's fall within accepted levels, production ageing may begin. If not, training on previously accepted and completed samples is necessary. After successful recalibration, production ageing commences. After a determined time or sample interval, readers should be retested. If retest results are not satisfactory, samples assigned age since the last successful test should be repeated after the reader recalibrates successfully.

After concluding production ageing, readers should be tested on a subsample of the just completed sample (100) and a subsample of 100 from the reference collection. Workshop participants recommended that bias plots and CV's (as noted in Campana et al. 1995), as well as percent agreement be used for reader evaluation. The magnitude of acceptable bias, CV, and percent agreement values were not determined at this workshop. The Canada DFO uses a reference collection of about 250 samples. The reference collection is subsampled (N=100), stratified by length. The quality standards to accept winter flounder ages require 75% percent agreement with no bias.

The Yellowtail Ageing Workshop (Walsh and Burnett 2002) did not set specific requirements and noted that each species, stock, and age structure is likely to have different requirements. These participants recommended that annual additions to the reference collections could be drawn from the most recently monitored subsample. The ASMFC Winter Flounder Ageing Workshop participants concluded that reference collections should be dynamic and large enough to prevent the potential problem of memorization.

2. Provide a report card (bias, CV's by age) with ageing results. Workshop participants recommend the use of the Connecticut DEP quality scale.

Assessment biologists should have quantitative data available to support readers' confidence level with respect to otolith and scale readings. The Connecticut Department of Environmental Protection assigns scores rating the quality or confidence in age interpretation of each otolith:

1= perfect
2= only one reasonable interpretation, though all marks not completely clear
3= two reasonable interpretations possible
4= no age assigned, more than two interpretations possible.

Any samples with scores of 3 or 4 require further processing and examination (otolith sectioned), use of alternate structures (scales), and/or re-examination by an experienced reader. Samples that are unclear or have confounding patterns to which an age cannot be confidently assigned should be omitted. Age/length key outliers can be identified by use of varied statistical and graphical auditing techniques.

3. Workshop participants recommend both scales and otoliths should be collected from specimens when possible.

Based on this and other studies, excellent agreement exists on ages derived from scales and otoliths from fishes less than age 6. Several laboratories utilize scales as the structure of choice, while others use otoliths. This recommendation allows for consistency with historical collections/databases and cost savings associated with laboratories purchasing equipment to age structures they routinely do not work with.

4. A study should be undertaken to compare ages interpreted from scales and whole/sectioned otoliths for older fish (5+).

Workshop participants believed it would be prudent to undertake this study, as relatively few specimens (either scales or otoliths) greater than age five have been examined in this manner.

5. Ageing workshops (formal/informal) should be held on a regular basis.

a) frequency of 3 to 5 years

b) in conjunction with similar species

Workshop participants believed that routine re-evaluation of aging structures and techniques would be appropriate and beneficial.

6. Adequate time should be provided between requests for age data and the assessment schedule.

Workshop participants agreed that stock assessment biologists should provide detailed data requests well in advance of an assessment, to include temporal and spatial ageing structure needs, as well as a size range and numbers of samples to collect. Mobilizing the appropriate manpower to collect large numbers of ageing structures in a short period of time may not be possible, especially if the structures are from larger animals or from multiple gear types/geographic areas. Funding to direct such effort may not be available on short notice. In all cases, the time required to physically prepare and age the samples must be taken into account.

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APPENDIX I Evaluation of the Consistency of Age Interpretation of Winter Flounder Otoliths Among Four Laboratories

by

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August 17, 2001

Introduction

Three stocks of winter flounder (Gulf of Maine, Georges Bank, and Southern New England/ Mid-Atlantic) are managed in the Northeast Groundfish Fishery Management Plan and ASMFC winter flounder Fishery Management Plan. At present age-base analytical assessments are conducted for the Georges Bank and Southern New England/ Mid-Atlantic stock. The Gulf of Maine stock's assessment is index based and mortality is estimated from catch curve analysis of survey data using ages 4-7.

In 1998, four laboratories (Connecticut DEP, Massachusetts DMF, New Jersey FGW, and NEFSC) read winter flounder hard parts (scales and otoliths) for construction of age length keys used in analytical assessments. A comparative exchange of 27 otoliths between Connecticut and New Jersey age readers in 1998 resulted in low agreement overall (56%) with highest agreement occurring on young fish (ages 1-3). Agreement on ages 4+ was 29% with evidence of relative bias on older fish. This conclusion led to a call for a larger exchange study among the four laboratories in order to evaluate the consistency of age interpretation among laboratories.

A two-part study comparing the age interpretations of the four laboratories was developed. The first part has been completed and is based on reading whole otoliths. The second part of the study, yet to be completed, will be based on the same set of samples using sectioned otoliths. We report on the results of the first part of the study.

Methods

The exchange consisted of 202 winter flounder otoliths samples from the Connecticut DEP 1998 spring trawl survey (April 1-May, 1998), Massachusetts' DMF 1997 spring survey (May 1998), NEFSC winter survey (February 7-27, 1998), and New Jersey spring surveys (March-May 1998). Participants are listed in Appendix I. Each age sample was coded with a random number from 100 to 302. Age readers provided age interpretations independent of other lab's interpretations. Sample numbers in each comparison differ from 202 total structures provided in the exchange due to the omission of samples deemed unreadable. These samples cover the broad geographical distribution of winter flounder, and geographical origin of the sample was unknown to the reader. In addition, readers were apprised of the dates of each survey, but were not aware of the date that individual samples were taken. Each envelope contained either 1 or 2 otoliths. Total length but not sex of each sample was available to the lab.

Participants were instructed to read whole otoliths using their laboratory methodology and ageing criteria (Appendix II). However, scales were not provided for the one participant who typically relies on scales as a primary structure and only uses whole otoliths as confirmation or backup. They were also asked to assign a quality index rank to each otolith: 1= excellent, 2= good, 3= difficult to interpret, 4= unreadable.

All samples with paired readings were included in the analysis regardless of quality index. For each paired comparison, one reader's interpretations were treated as "control " and the other reader's interpretation the "variable ". Since true ages are unknown, results are relative and these analyses only measure consistency in interpretation among readers. Precision and bias were analyzed using statistical and graphical methods found in Campana et al. (1995). Precision was measured using percent agreement, mean age with 95% confidence intervals, and coefficient of variation. For each paired reading of a sample, coefficient of variation was calculated using:

$$CV_j = 100 \times \frac{\sqrt{\frac{\sum_{i=1}^{R}(X_{i,j} - \overline{X}_j)^2}{R-1}}}{\overline{X}_j}$$

Mean CV's were calculated for each of the control's age groups and all age groups for each paired comparison.

The presence of bias was tested using regression analysis. The null hypothesis for the regression line was slope =1 and intercept=0. Significant deviations from either parameter indicate bias between readers. Age bias plots were also examined for bias. Systematic deviation from the 1:1 equivalency line also indicates bias. Samples used in this study were not age validated; therefore measuring accuracy was not possible.

Results

Reader 1 vs. Reader 2.

Overall percent agreement was 77% (N=186) with an overall coefficient of variation of 3.9% (Table A.1. 1). Percent agreement was relatively high for ages 1-4, but declined with age. Percent agreement for 4^+ was 71%. The age bias plot and age frequency plot suggested a slight bias beginning at age 6 (Figure A.1. 1 and Figure A.1. 2). The regression's slope was significantly different than 1 ($P<0.001$) and the intercept was significantly different than zero ($P<0.01$), indicating the presence of bias (Table A.1. 2). The bias is primarily being driven by a tendency for reader 2 to overage at the younger ages and underage at ages > 6 relative to reader 1. Precision decreases and bias increases at ages 8^+, but results are based on only 12 samples.

Table A.1. 1: Descriptive statistics derived from the comparison of reader 1 and reader 2.

Reader 1 Age	Reader 2 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.13	0.90 - 1.35	5.9	87.5	8
2	2.23	2.05 - 2.40	6.1	80.6	31
3	3.13	2.94 - 3.31	2.1	93.8	32
4	4.10	3.97 - 4.22	2.3	88.1	42
5	5.09	4.90 - 5.27	4.4	68.6	35
6	5.83	5.60 - 6.06	3.5	72.2	18
7	6.88	6.33 - 7.42	6.4	37.5	8
8	7.63	7.29 - 7.96	3.5	62.5	8
9	8.00	8.00 -8.00	8.3	00.0	4
All ages			3.9	77.4	186

Table A.1. 2: Regression of reader 2 age interpretation on reader 1 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probability slope=1
186	0.44 ± 0.19	<0.001	0.90 ± 0.04	< 0.001

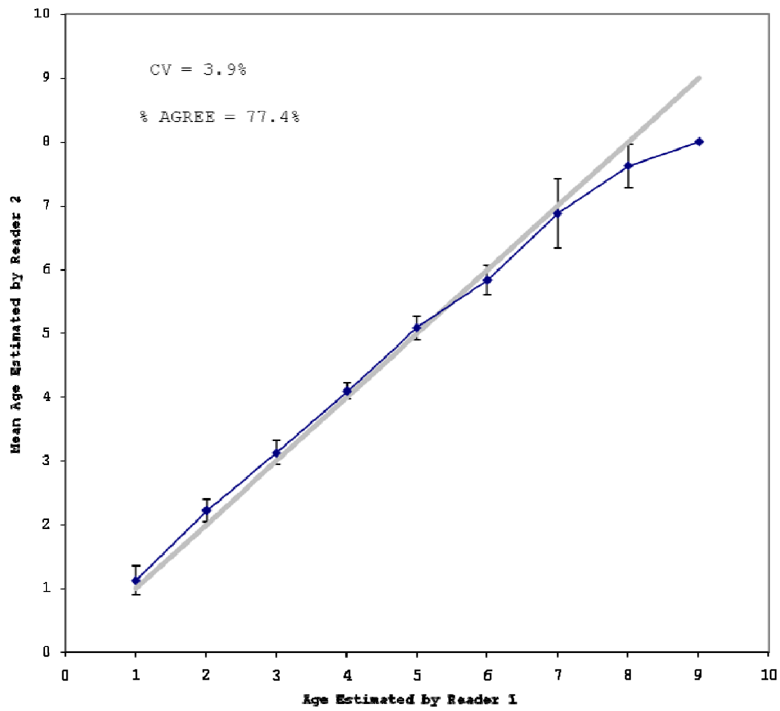

Figure A.1. 1: Bias plot from the comparison of readers 1 and 2. Solid line represents the 1:1 equivalency line. Dots represent mean age of reader 2. Error bars represent 95% confidence intervals.

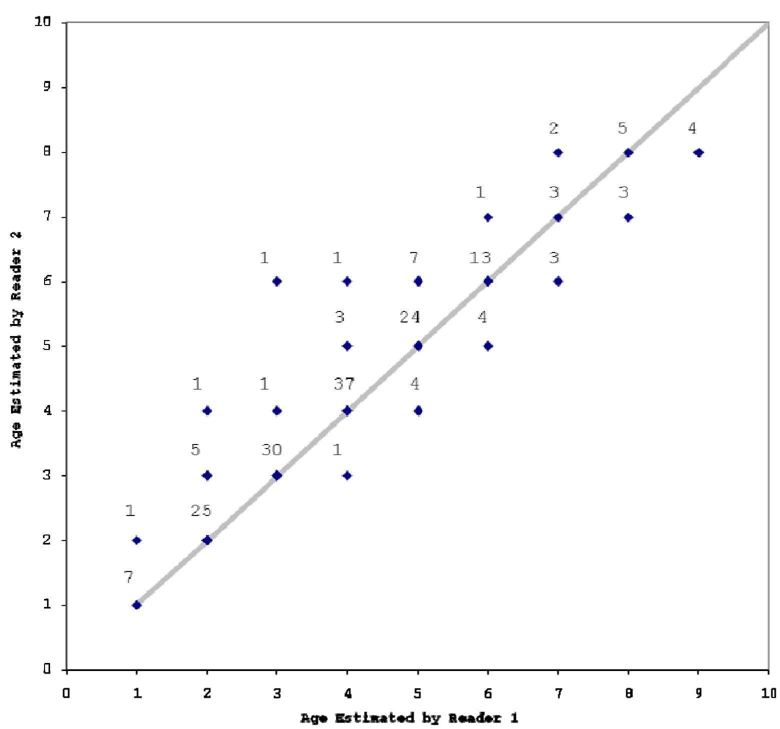

Figure A.1. 2: Age frequency plot from the comparison of readers 1 and 2.

Reader 1 vs. Reader 4.

Overall percent agreement was 32% (N=187) with an overall coefficient of variation of 20.9% (Table A.1. 3). Percent agreement by age showed little pattern. The age bias plot and age frequency plot suggest considerable bias with wide confidence intervals around the mean age (Figure A.1. 4 and Figure A.1. 3). The regression's slope was significantly different than 1 (P<0.01) and the intercept was significantly different than zero (P<0.001), indicating the presence of bias (Table A.1. 4). Low precision and bias indicates poor consistency of interpretation between these two readers.

Table A.1. 3: Descriptive statistics derived from the comparison of reader 1 and reader 4.

Reader 1 Age	Reader 4 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.75	1.29 - 2.21	32.4	38	8
2	2.94	2.67 - 3.20	24.3	26	31
3	4.00	3.53 - 4.47	16.2	51	35
4	6.31	5.48 - 7.14	27.1	29	42
5	6.80	5.93 - 7.67	19.7	29	35
6	7.39	6.62 - 8.16	17.1	6	18
7	8.43	7.12 - 9.73	17.9	14	7
8	8.57	7.27 - 9.88	9.2	57	7
9	9.25	8.83 - 9.67	1.9	75	4
Total			20.9	32	187

Table A.1. 4: Regression of reader 4 age interpretation on reader 1 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probability slope=1
187	1.70 ± 0.43	<0.001	0.43 ± 0.07	<0.001

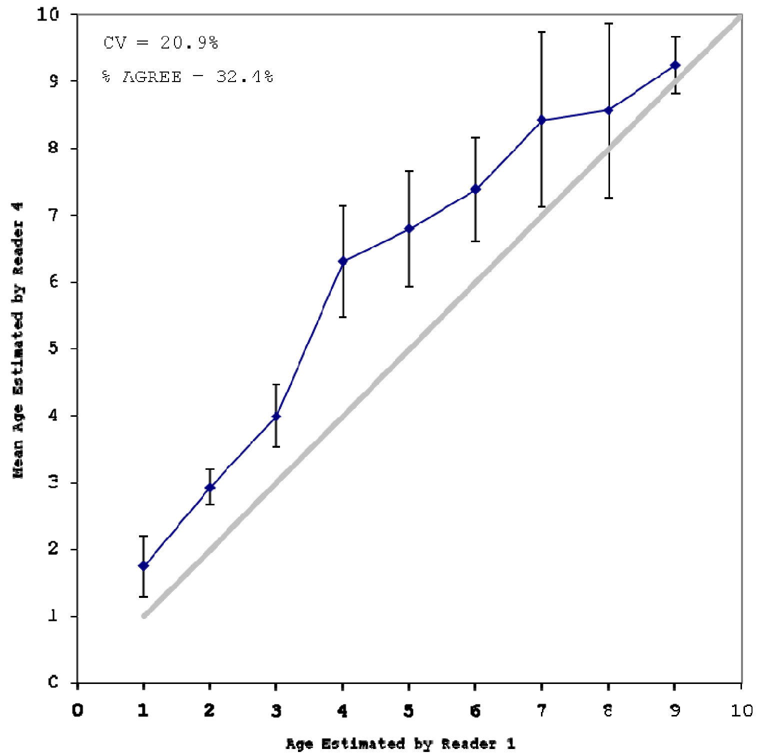

Figure A.1. 4: Bias plot from the comparison of readers 1 and 4. Solid line represents the 1:1 equivalency line. Dots represent mean age of reader 4. Error bars represent 95% confidence intervals.

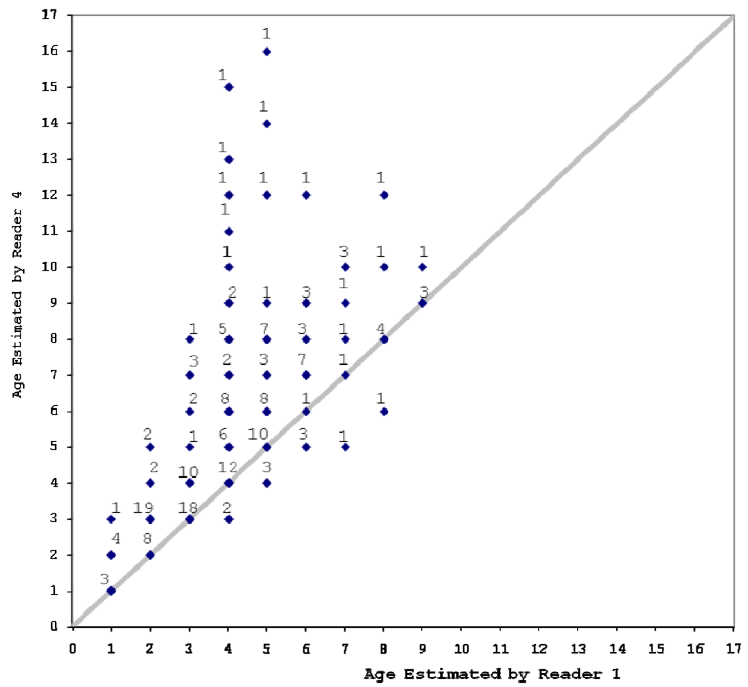

Figure A.1. 3: Age frequency plot from the comparison of readers 1 and 4.

Reader 2 vs. Reader 4.

Overall percent agreement was 35% (N=196) with an overall coefficient of variation of 19.0% (Table A.1. 5). Percent agreement was relatively low at all ages with no discernible pattern. Percent agreement of age 4+ was 28%. The bias plot and age frequency plot (Figure A.1. 5 and Figure A.1. 6) suggest bias at all ages and wide confidence intervals at ages 4 and above. The regression's slope was not significantly different than 1 (P<0.10) but the intercept was significantly different than zero (P<0.05), indicating the presence of bias (Table A.1. 6). Low precision and bias indicates poor consistency of interpretation between these two readers.

Table A.1. 5: Descriptive statistics derived from the comparison of reader 2 and reader 4.

Reader 2 Age	Reader 4 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.57	1.20 - 1.94	26.9	42.9	7
2	2.78	2.52 - 3.04	20.8	33.3	27
3	3.68	3.35 - 4.00	13.1	55.0	40
4	6.20	5.40 - 7.00	26.4	31.1	45
5	6.57	5.83 - 7.31	16.6	37.1	35
6	7.92	7.06 - 8.77	19.1	12.5	24
7	8.43	7.32 - 9.54	14.9	14.3	7
8	8.73	7.82 - 9.64	10.4	27.3	11
9					
Total			19.0	34.7	196

Table A.1. 6: Regression of reader 2 age interpretation on reader 4 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probability slope=1
196	0.78± 0.75	<0.05	1.14 ± 0.17	<0.10

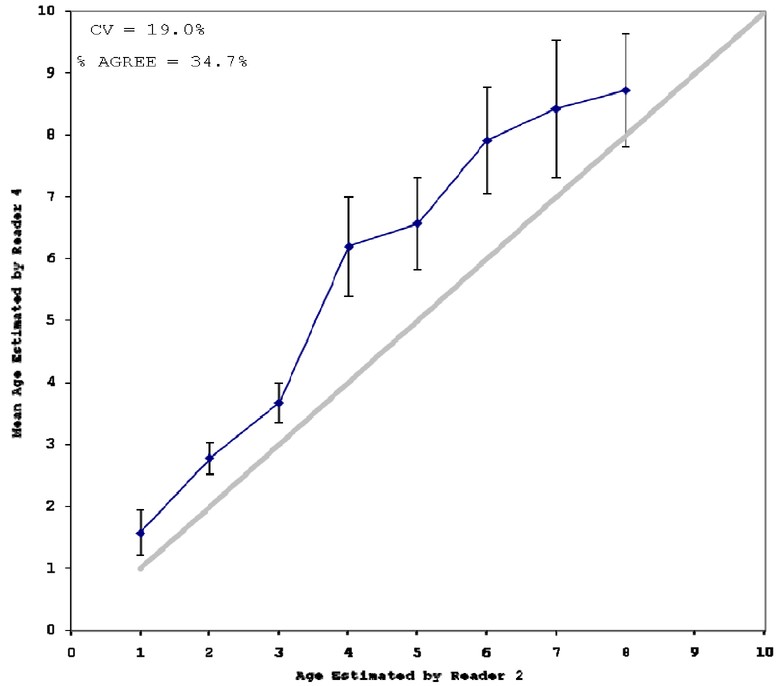

Figure A.1. 5: Bias plot from the comparison of readers 2 and 4. Solid line represents the 1:1 equivalency line. Dots represent mean age of reader 4. Error bars represent 95% confidence intervals.

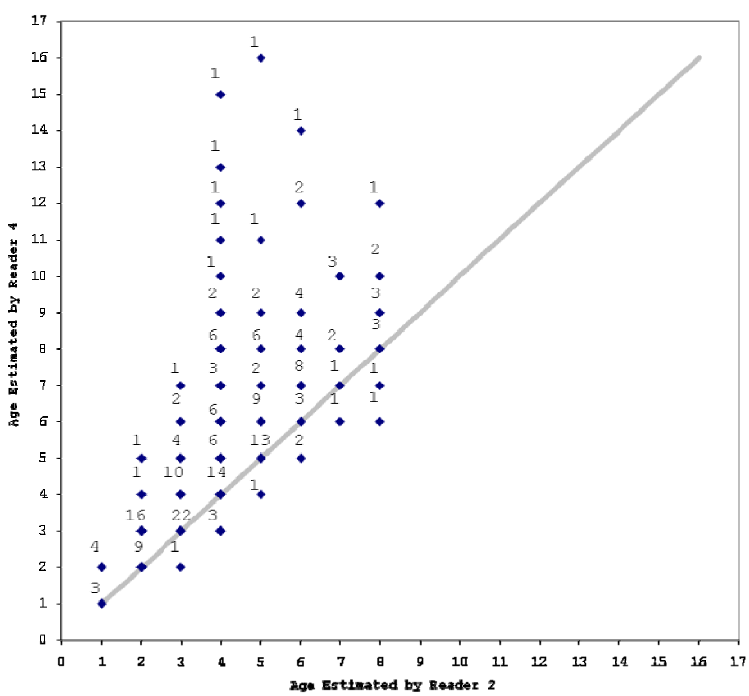

Figure A.1. 6: Age frequency plot from the comparison of readers 2 and 4.

Reader 3 vs. Reader 1.

Overall percent agreement was 76.6% (N=188) with an overall coefficient of variation of 4.4% (Table A.1. 7). Percent agreement was relatively high for ages 1-3, but declined with age. Percent agreement for age 4^+ was 65%. The bias plot and age frequency plot suggested bias beginning at age 4 and the magnitude of bias increasing with age (Figure A.1. 7 and Figure A.1. 8). The regression has a slope significantly different than 1 (P<0.05) and the intercept was significantly different than zero (P<0.01), indicating the presence of bias (Table A.1. 8). Age interpretations are consistent for ages 1-3 but inconsistent beyond age 3.

Table A.1. 7: Descriptive statistics derived from the comparison of reader 3 and reader 1.

Reader 3 Age	Reader 1 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.00	1.00 - 1.00	0.0	100.0	7
2	1.97	1.91 - 2.03	1.5	96.9	32
3	3.15	3.02 - 3.29	3.0	87.2	39
4	4.29	4.13 - 4.45	5.0	73.1	52
5	5.56	5.23 - 5.89	6.5	67.6	34
6	6.67	6.12 - 7.21	8.2	53.3	15
7	7.57	7.20 - 7.94	5.4	42.9	7
8	9.00	9.00 - 9.00	8.3	00.0	2
9					
Total			4.4	76.6	188

Table A.1. 8: Regression of reader 3 age interpretation on reader 1 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probability slope=1
188	0-0.30 ± 0.26	<0.05	1.15 ± 0.06	<0.01

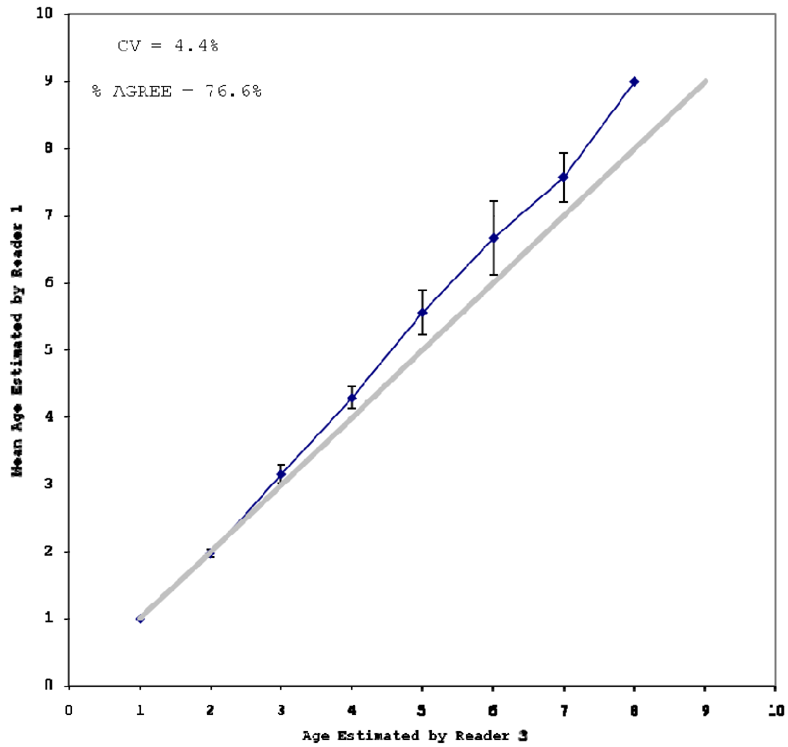

Figure A.1. 7: Bias plot from the comparison of readers 3 and 1. Solid line represents the 1:1equivalency line. Dots represent mean age of reader 4. Error bars represent 95% confidence intervals.

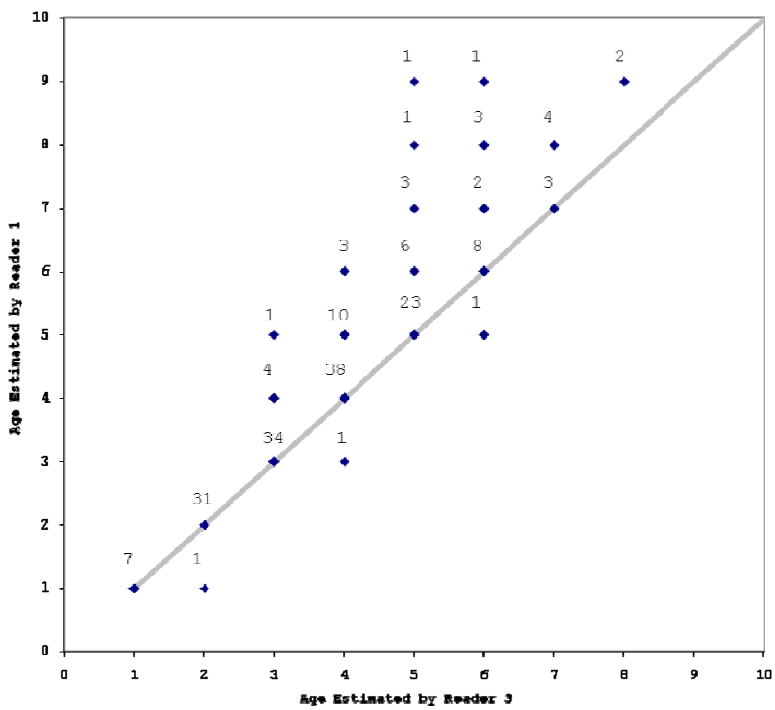

Figure A.1. 8: Age frequency plot from the comparison of reader 3 and 1.

Reader 3 vs. Reader 2.

Overall percent agreement was 69% (N=196) with an overall coefficient of variation of 6.2% (Table A.1. 9). Percent agreement was relatively high for ages 1-3, but declined with age. Percent agreement for 4^+ was 59%. The bias plot and age frequency plot suggested bias at all ages excepting 1 and 8 (Figure A.1. 9 and Figure A.1. 10). The magnitude of bias increased beginning at age 4. The regression has a slope significantly different than 1 (P<0.05) but the intercept was not significantly different than zero (P=0.84), indicating the presence of bias (Table A.1. 10). Age interpretations are consistent at younger ages, but consistency declines beyond age 3.

Table A.1. 9: Descriptive statistics derived from the comparison of reader 3 and reader 2.

Reader 3 Age	Reader 2 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.00	1.00 - 1.00	0.0	100.0	7
2	2.24	2.07 - 2.40	6.4	79.4	34
3	3.15	3.04 - 3.27	3.1	84.6	39
4	4.46	4.26 - 4.67	7.4	64.3	56
5	5.39	5.13 - 5.65	6.3	61.1	36
6	6.67	6.27 - 7.07	6.9	53.3	15
7	7.57	7.03 - 8.11	8.3	14.3	7
8	8.00	8.00 - 8.00	0.0	100.0	2
9					
Total			6.2	69.4	196

Table A.1. 10: Regression of reader 2 age interpretation on reader 3 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probabilit y slope=1
196	0.03± 0.26	<0.84	1.1 ± 0.06	<0.001

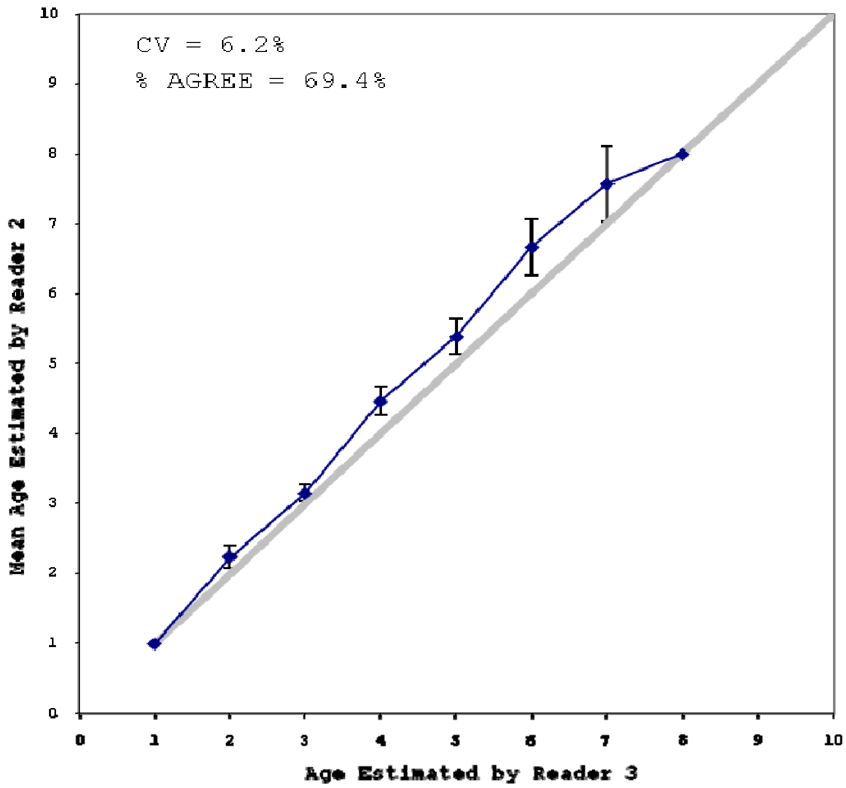

Figure A.1. 9: Bias plot from the comparison of readers 3 and 2. Solid line represents the 1:1 equivalency line. Dots represent mean age of reader 2. Error bars represent 95% confidence intervals.

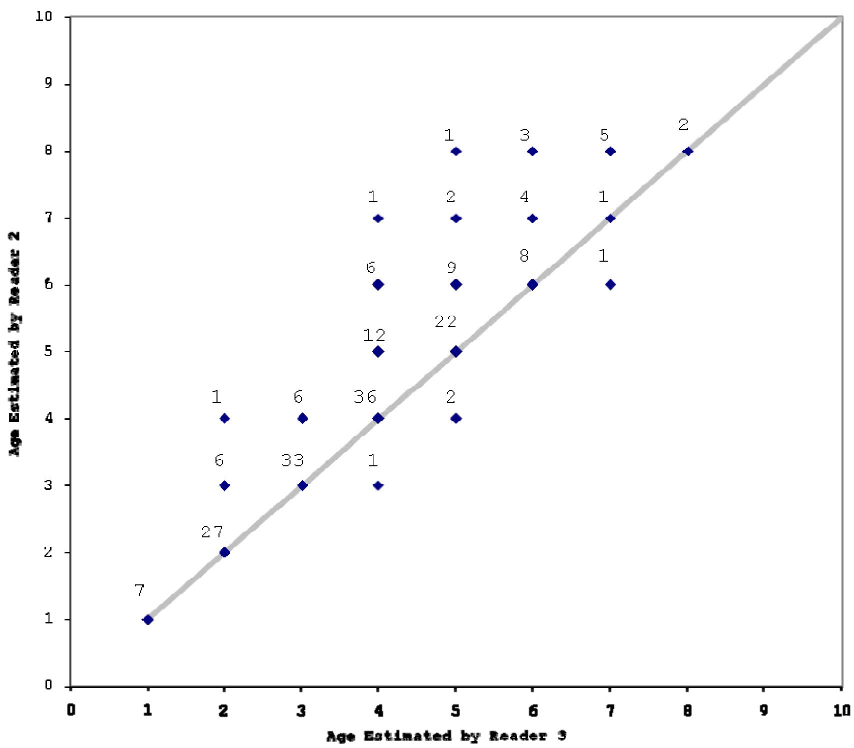

Figure A.1. 10: Age frequency plot from the comparison of readers 3 and 2.

Reader 3 vs. Reader 4

Overall percent agreement was 29% (N=198) with an overall coefficient of variation of 23.3% (Table A.1. 11). Percent agreement showed a slight tendency to decline at age. The bias plot and age frequency plot suggest considerable bias with wide confidence intervals around the mean age at all age comparisons (Figure A.1. 11 and Figure A.1. 12). The regression's slope was significantly different than 1 (P<0.01) but the intercept was not significantly different than zero (P<0.001), indicating the presence of bias (Table A.1. 12). Low precision and bias indicates poor consistency of interpretation between these two readers.

Table A.1. 11: Descriptive statistics derived from the comparison of reader 3 and reader 4.

Reader 3 Age	Reader 4 Mean age	95% CI	Mean CV	Percent agreement	Sample size
1	1.57	1.20 - 1.94	26.9	43	7
2	2.97	2.69 - 3.25	24.8	26	34
3	4.14	3.57 - 4.71	18.0	50	42
4	6.36	5.73 - 6.98	27.4	23	56
5	7.08	6.34 - 7.83	21.7	31	36
6	9.27	8.26 - 10.27	28.5	0	15
7	7.83	6.86 - 8.80	10.7	17	6
8	9.50	8.81 - 10.19	12.0	0	2
9					
Total			23.3	29	198

Table A.1. 12: Regression of reader 4 age interpretation on reader 3 ages.

N	Intercept and 95% confidence interval	Probability intercept=0	Slope and 95% confidence interval	Probability slope=1
198	0.46± 0.80	<0.26	1.34 ± 0.20	<0.001

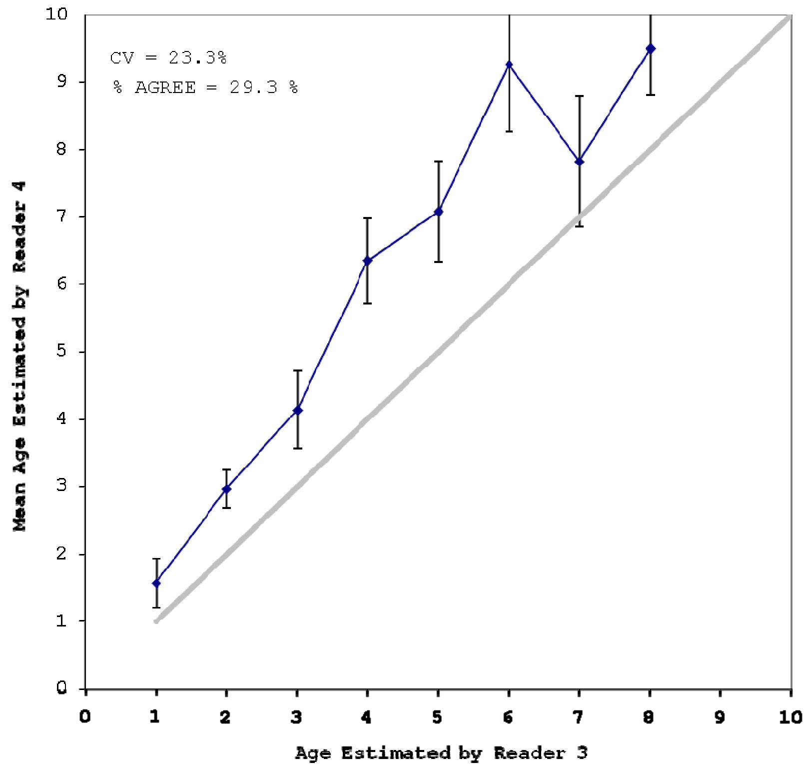

Figure A.1. 11: Bias plot from the comparison of readers 3 and 4. Solid line represents the 1:1 equivalency line. Dots represent mean age of reader 4. Error bars represent 95% confidence intervals.

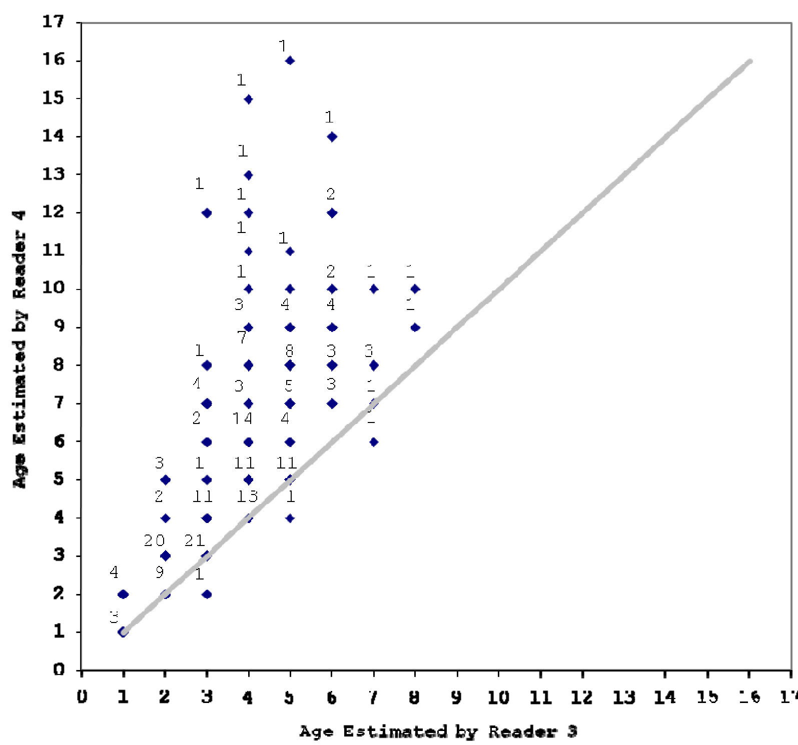

Figure A.1. 12: Age frequency plot from the comparison of readers 3 and 4.

Conclusions

These analyses only pertain to consistency in interpretation among the four readers. Bias as measured by this study relates to systematic differences detected within comparisons between readers and does not address accuracy relative to true age. Overall, these results suggest that interpretations among the four readers are inconsistent, especially on 4^+ fish, which are considered fully recruited. The inconsistency consists of low percent agreement, low precision, and bias. Reader 4 interpretations were very inconsistent with readers 1, 2 and 3. The results suggest that reader 4's interpretation of the first annulus and subsequent annuli is markedly different from the other readers. Consistency was higher among readers 1-3, but even the best comparison (readers 1+ 2) had evidence of bias. Results suggest good consistency in interpretation of the first annulus among readers 1 – 3. However, inconsistency, especially bias, increased with age, suggesting either heterogeneous criteria among readers for discriminating spawning checks from annuli and/or interpretation of the edge type (hyaline or opaque) among these readers.

The inconsistency among readers identified in this study may be somewhat inflated compared with inconsistency occurring within labs. Ages were assigned without the option of sectioning difficult otoliths or the use of scales (the primary structure typically used by one reader). In addition, age readers may have felt psychological pressure to provide an age interpretation on an age structure whose condition normally would have resulted in no age assignment. Finally, readers were asked to read structures from fish taken outside their geographical area or during a slightly different time-period. Interpreting ages from fish with unfamiliar growth patterns or blindness to the collection date could inflate inconsistency.

Further analysis of current data or completion of the proposed second part of this study could help delineate factors contributing to inconsistency among readers. These include examining for sex, seasonality, geographic and age structure quality effects.

These results suggest the need for an age and growth workshop for winter flounder. Reaching consensus on criteria for defining annuli and interpretation of edge type should be the primary objective of the workshop. Use of other structures (e.g., scales) or methodology (e.g., sectioning) to improve consistency should also be examined. Finally, the group should develop recommendations for monitoring and maintaining consistency among age readers in the future. These recommendations could include future exchanges or development of reference collections to measure accuracy and precision of production age determination.

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Appendix AI.2 Participants' Laboratory's Methodologies

In the following methods, transmitted light is defined as light transmitted through the otolith from a light source below the otolith. Reflected light refers to a light source reflected from the surface of the otolith with the light source shining down on the top surface of the specimen.

Connecticut DEP

Connecticut DEP ages winter flounder using whole otoliths mounted in water. Otoliths were mounted in water and viewed with reflected light. All were assumed to have had their birthdays on April, so an age was assigned to an edge area even if no new growth (opaque/white material for otoliths viewed with reflected light) was present.

Massachusetts DMF

Both scales and otoliths are placed in a water-filled watch glass and aged through a dissection scope at up to 3X using both transmitted light as well as varying amounts of transmitted source light reflected by a mirror through the scale or otolith as necessary to highlight growth zones. The Massachusetts age reader relies on scales because of more consistent clarity. Whole otoliths are checked as confirmation of the scale age or relied upon when most scales in the sample are regenerated. The reader finds whole otoliths generally more difficult to read after age 5 or so because of thickness and varying clarity.

New Jersey DFGW

New Jersey ages winter flounder using whole otoliths. Whole otoliths are mounted in water and read using reflected light through a microscope. Magnifications are generally 2X. Opaque rings appear white and translucent rings appear dark. One opaque ring and one translucent ring equals one year.

NEFSC

Whole otoliths aged by the NEFSC were viewed against a dark background at magnifications of 6-12 X using reflected light. Mild soapy water was applied as necessary to enhance otolith clarity. An annulus was considered to be a completed hyaline zone (i.e. a hyaline zone bounded by opaque material). For otoliths from older fish, it was often necessary to re-orient the otolith from the flat position in order to see annuli accumulated on the otolith edge. Age determinations were based upon the number of annuli and interpretation of the edge type and increment.

Appendix AI.3 Life history and fishery information for three stocks of winter flounder

Southern New England/ Mid-Atlantic stock complex is distributed on the shelf from south and east of Cape Cod to New Jersey, shoreward of the Great south channel. For females, the age of 50% maturity is 3.0 years (O'Brien ET al, 1993). The assessment uses ages 1-6 with a 7+ plus group (NEFSC 1999). In recent years, fish are partially recruited beginning at age 2 and are fully recruited at age 4. The fishery is mostly commercial with otter trawls accounting for 95% of the commercial landings. In recent years, recreational catch accounted for approximately 20% of the total landings

The Georges Bank winter flounder stock is distributed on the shallower portions of Georges Bank. Georges Bank winter flounder have higher growth rates than the inshore stocks. For females, the age of 50% maturity is 1.83 years (NEFSC, 1999). In the most recent years, Georges Bank winter flounder begin to recruit to the fishery at age 2 and are fully recruited at age 4. The assessment uses ages 1-6 with a 7+ age group. Recreational catches are insignificant and 95% of the commercial landings are by otter trawl.

The Gulf of Maine winter flounder stock is distributed in shallow coastal waters from Cape Cod Bay to Nova Scotia (NEFSC 1995). Winter flounder in Massachusetts' waters north of Cape Cod grow slower and mature later than winter flounder in Massachusetts' waters south and east of Cape Cod (Witherell and Burnett, 1993). For females, age of 50% maturity was 3.3 years. The Gulf of Maine winter flounder assessment is index-based with estimates of mortality derived from survey catch curve analysis using ages 4-7 (Cadrin et. al. 1996). The stock is exploited by commercial and recreational fisheries. Recreational landings were a significant proportion of total landings in the early 1980's, but recreational landings as a proportion of total landings have markedly declined in recent years. Commercial landings are harvested with otter trawls and gillnets.

APPENDIX II Draft Agenda for ASMFC Winter Flounder Ageing Workshop

August 22-23, 2001 Northeast Fisheries Science Center - Woods Hole, MA

I. Terms of Reference

- Evaluate the various structures and methods to age winter flounder
- Develop standardized protocols for ageing, training, testing, and evaluating consistency
- Produce written report of Workshop Proceedings

II. Wednesday August 22 Aquarium Conference Room

0900-0915	Welcoming remarks	
0915-1000	Introduction	(Jay Burnett)
	Exchange results	(Steve Correia)
	Statement of problem	(Steve Correia)
	Terms of Reference	(Steve Correia)
	Adopt Agenda	(Steven Correia)
	Winter flounder biology	(Jay Burnett)
	Ageing and age validation	(Jay Burnett, Ruth Haas-Castro)
1000-1020	Coffee Break	
1020-1120	Review of regional biology	(Participants)
	Review of ageing	(Participants)
1120-1215	Review of terminology/conventions	(Jay Burnett)
	Review of processing methods	(Jay Burnett)
1215-1345	Lunch	
1345-1630	"Hands on" ageing session	(All)
	Scales, whole otoliths, sectioned otoliths	
1630-1700	Discussion and Summary	(Steve Correia, Jay Burnett)
1700	Workshop Report	(Steve Correia, Jay Burnett)
1800	Social hour	

III. Thursday August 23 Aquarium Conference Room

0830-0845	Review, plan for Day 2	(Jay Burnett)
0845-1000	"Hands on" ageing session	(All)

1000-1020	Coffee Break	
1020-1100	"Hands on", continued	(All)
1100-1230	ASMFC reference collection	(Jay Burnett)
	Future exchanges	(Steve Correia)
	Develop recommendations	(Steve Correia)
	Future plans	(Steve Correia)
	Discussion and summary	(Steve Correia)
1300	Adjourn formal workshop	(Steve Correia)
1400-1700	Workshop Report	(Steve Correia, Jay Burnett)
	Informal "hands on" ageing session	(All)

APPENDIX III Evaluation Summary of the ASMFC Winter Flounder Ageing Workshop

August 22-23, 2001 Northeast Fisheries Science Center - Woods Hole, MA

Workshop participants were strongly encouraged to complete an evaluation form. Nine evaluations were received. The age reading experience of those respondents ranged from novice to expert.

1. What were the most useful aspects of the workshop?

- Hands-on stuff
- Review of whole otolith ageing
- Hands on sessions with discussion on ages
- Terminology and convention
- Bringing together those who age the same species to compare methods and interpretations
- "Hands on" otolith ageing. View of various laboratory protocols.
- Reaching consensus on ageing the particular species and having more than 1 structure to age with for examples (oto's-whole, sectioned & scales). Having both experienced and inexperienced readers present. Having many regions represented.
- Meeting with expert agers and going over otoliths hands on. Seeing the results of the comparison study.
- Listening to the interpretations of age samples by experienced age readers

2. What did you find to be the least useful aspect of the workshop?

- Formal presentations
- Would liked to have had more discussion of Federal/ State role of this activity 5-10 years out. Is it cost effective (given noted uncertainty and bias) for more people/agencies to be involved? Why not an ageing center or enhance NEFSC capabilities through budget appropriations?
- Not applicable
- Too much detail on exchange regressions
- Being a new reader I found all useful. As discussions developed or went off track I continued to learn all new aspects of ageing & species
- It was all useful

3. For each of the following agenda items, please rank the amount of time spent on each using the following scale: 1= should be eliminated, 2= should be covered but give less time, 3= just right, 4= more time needed, 5 much more time needed.

Agenda Item	1 should be eliminated	2= should be covered but give less time,	3= just right	4= more time needed	5 much more time needed
Review of exchange results					
Review of Winter flounder biology					
Review of ageing and validation studies					
Review of regional biology					
Review of terminology and conventions					
Review of ageing procedures					
Hands on sessions					
Discussion and summary					
Reporting Workshop results					

See Chairperson's evaluation for summary of the results of this table.

4. How would you improve the "hands-on" workshop?

- Smaller groups with more work stations
- Attempt to find a way to simultaneously view (through projection) two or more structures. Insure all principal players/labs are present.
- Would (?); maybe put ages down on paper to be able to see the percent agreement
- A bit more time, presentation by each reader/ region that showed representative structures and how interpreted
- Run through sectioning methods
- Have more initial info on species so everyone comes in on even page with those more experienced. Maybe send out info early to bring everyone up to a certain working level. Allow more time for inexperienced to review some structures to age on their own time.
- More work for individuals to look at structures alone and then be critiqued
- Project images of scales, otoliths, and sectioned otoliths from same fish and/or make sections of otoliths when called for if possible

5. Did the workshop complete its terms of reference or is more work needed?

- Essentially yes
- Yes… but now the results must be communicated/sent to and assimilated by NJ lab. I'm not confident the latter can be achieved by their ageing specialist without viewing all of what conference participants did.
- Yes. More work needed for reference collection
- More work needed
- I thought terms of reference were met
- Did well with time allotted.
- Completed
- I think the workshop went quite far in meeting the terms of reference. I do believe that the limited time left some loose ends in standardized protocols, particularly for training.

The following questions relate to your willingness to participate in future studies. Please identify your laboratory/ agency.

Laboratory/ agency ____

Name____

1. Would your lab participate in developing a reference collection? yes_6_ no_0__ maybe_1_

2. Would your lab participate in a future study to compare :
   a. whole otoliths and sectioned otoliths? yes__3__ no_1__ maybe___3_ _
   b. Scales to whole otoliths? yes___4_ no _0_ maybe___3_ _
   c. Whole otoliths, sectioned otoliths, and scales? yes__2_ no_1__ maybe __4__

3. Would your lab be willing to participate in future exchanges? yes_6__ no__0_ maybe__1_

Table A.III.1**:** Summary of answers to questions 1-3 by organization

	NEFSC	MADMF[1]	DFO	CT DEP	RI FGW	NHFG	ME DMR
Participation in developing a reference collection	yes	yes	yes	yes	maybe	yes	yes
Participation in a future study to compare:							
whole otoliths and sectioned otoliths	yes	no	yes	yes	maybe	maybe	maybe
scales to whole otoliths	yes	yes	maybe	yes	maybe	yes	maybe
whole otoliths, sectioned otoliths, and scales	yes	no	maybe	yes	maybe	maybe	maybe
Willingness to participate in future exchanges	yes	yes	yes	yes	maybe	yes	yes

[1]Massachusetts Division of Marine Fisheries does not have otolith sectioning equipment, hence the negative reply to participating in studies involving sectioned otoliths.

4. Any comments regarding participation in and/or recommendations for future studies, workshops, etc. (Chairman comments are in parentheses).

- As always, time and resources permitting. Careful planning is of the essence.
- **No equipment to section (this person answered no regarding participating in whole otolith, sectioned otolith, and whole otolith-scale-sectioned otoliths with asterisks. They would participate if training/time was available)*
- **of course this would have to be cleared with Supervisor Management. But I'm very interested in participating (Asterisk on yes answers to willingness to participate in developing a reference collection, whole otolith-sectioned otoliths and future exchanges).*
- Our growth and ageing lab is in its beginning stages. I would be willing to participate in exchanges and the reference collection but would feel more comfortable getting more experience in otolith reading first.
- Maybe in conjunction with Maine (refers to developing a reference collection)
- Small number of samples due to limited personnel with NH Marine Division.
- Future workshops - Bring all methods of preparation to workshop. Allow ageing reviews with all methodologies.
- In conjunction with New Hampshire (refers to developing a reference collection).
- Workshops on other species of groundfish would be helpful to me.
- I suggest that time may have been saved if pertinent protocols and recommendations from previous age workshops were presented as starting point for discussion from which parts could be adopted, modified, or rejected.

Chairperson's evaluation

The workshop's terms of references were:

Term of Reference 1: Evaluate the various structures and methods to age winter flounder

This term of reference was included in the workshop in order to identify the source of variation and bias in interpretation among laboratories identified in the evaluation study (Correia and King 2001). This term of reference has been met. This portion of the workshop could have been accomplished using just the expert readers involved in the evaluation study. The amount of time of the workshop would have been about the same. The real test of whether this workshop achieved this primary objective will only occur after development of the reference collection and implementation of testing and evaluation protocols occurs. Absent these developments, we will not be able to evaluate the efficacy of this workshop.

Discussion concerning differentiating annuli from various checks, splits, etc, was useful for imparting the "art of interpretation" to inexperienced and non-age readers. This probably exposed many inexperienced readers to the fact that ageing interpretations involve observation of subtle and obvious patterns within a particular structure(s). Even the best of ageing manuals will be unable to express this aspect of ageing. Many ageing manuals present one or two criteria that should be rigidly followed while expert readers use pattern recognition derived from experience.

Term of Reference 2: Develop standardized protocols

Ageing

The workshop adequately defined the criteria for differentiating annuli from other marks. The workshop did not go into detail on methodology for sectioning otoliths. Lack of methodological detail was probably not important for experienced age readers, but was important for new age readers. The workshop was able to determine the adequacy of using whole otoliths, sectioned otoliths and scales based on consensus opinion and previous studies, but an analytical evaluation was not possible in the time available. A technical evaluation of various structures to determine precision and bias is better suited for studies held outside a workshop format. Some time may have been saved by reviewing standardized protocols for species similar to winter flounder and adopting those most appropriate for winter flounder. This term of reference could have been completed more efficiently using only experienced readers. If possible, future workshops should allow simultaneously projecting images of more than one structure (both otoliths, whole and sectioned otoliths, scales and otoliths) if comparisons of structures are an issue. This would have required either multiple image analysis systems if actual structures are used, or a good amount of preparation if only images are used.

Material used varied in readability from easy to difficult. This was useful in the development of criteria. Most time was spent on whole otoliths, followed by sectioned otoliths and least amount on scales. Adding another day to the workshop could have allowed a more in-depth study of these structures. For example, the group did not examine thin-sectioned otoliths using transmitted light.

Training
The workshop did not develop protocols for training. The workshop recommended developing reference collections, which are one of the best tools available for training. Reference collections allow the development of consistency standards (percent agreement, Coefficient of variation, bias) that could determine when a trainee is ready for production ageing. The workshop report will include annotated images that will be useful for training purposes.

As a training exercise, this workshop could have been improved by having more workstations involved. This is easier said than done and requires material. As an alternative, a network with multiple PC's would allow multiple stations without having scopes, etc would allow for multiple images to be used for training. However, the plane of focus in an image cannot be changed as they can in a microfiche reader or microscope. Microscopes other than teaching scopes with pairs of objectives is not useful for teaching as pointing out marks may be difficult. I suggest that pairing of experienced with several inexperienced readers may have improved this as a training exercise.

Although advantages exist for having inexperienced readers witness the development of consensus ages, a separate training workshop probably would be a more efficient use of time. I believe that insufficient time was available to have new readers propose age determination followed by expert commentary at this workshop. However about 1.5 hrs was available for hands-on work after the workshop was formally adjourned (3:30 PM) but was not utilized by any readers. One suggestion to consider at future workshops is to allow a short bit of time for everyone to add an age determination prior to discussion of a particular structure. Providing a sheet of paper with a blank table for age determination and notes would encourage readers to make an age determination. Standardized protocols/ ageing manuals should be developed prior to a training workshop.

Material examined in this workshop covered a wide geographic area (Southern Gulf of St Lawrence to coastal New Jersey including Georges Bank. Material also varied in readability. This was an asset to the workshop. One suggestion made by a participant would be to have experienced readers bring a set of material that was sorted by age and difficulty of interpretation. This would be especially useful in a training workshop.

Testing and Evaluating Consistency
The workshop recommends the development of reference collections. Implementing this recommendation would allow for testing and evaluating consistency on a regular basis. This would allow for early detection of problems before production ageing occurs. The workshop did not address values to use as benchmarks for acceptable ageing. Values cannot be set until more evaluation data are available to set standards. Developing consensus ageing for the reference collection at this meeting was not feasible given the multiple objectives of the workshop. With foresight, the beginning of the reference collection could have started at this workshop. I recommend that future workshop save images/ structures with consensus age. This aspect of the workshop would have only required the presence of experienced age readers.

Term of reference # 3: Produce written report of Workshop Proceedings

This term of reference in reality has two elements: a report relative to resolving differences found in the evaluation study and to form the basis of an ageing manual. In reality the process would have been better served to produce two separate reports. Perhaps ACCSP or ASMFC can adopt standardized terminology/birth date conventions that can be used by all future workshops. Terminology for this workshop was adopted directly from Penttila and Dery (1988). Similarly material regarding development of the reference collection was adopted from the report of the yellowtail ageing workshop (Walsh and Burnett 2002).

Time utilization

The agenda for this meeting was kept flexible and was adjusted as needed to meet the needs of the workshop. The evaluation sheet included section to evaluate time usage. Participants were ask to evaluate time spent on each agenda item using the following directions:

3. For each of the following agenda items, please rank the amount of time spent on each using the following scale: 1= should be eliminated, 2= should be covered but give less time, 3= just right, 4= more time needed, 5 much more time needed.

Summary statistics are presented in Table A.III.2.

Table A.III.2. Summary statistics on evaluation of time usage from evaluation sheets.

	N	mean	SD	CV	Range
Review of exchange material	**8**	**2.9**	**0.60**	**20.9**	**2-4**
Review of winter flounder biology	**8**	**3.3**	**0.88**	**27.1**	**2-5**
Review of ageing and validation studies	**8**	**3.5**	**0.49**	**14.1**	**3-4**
Review of regional biology	**8**	**3.4**	**0.90**	**26.8**	**2-5**
Review of terminology and conventions	**8**	**3.5**	**0.73**	**20.8**	**2-4**
Review of ageing procedures	**8**	**3.8**	**0.70**	**18.7**	**3-5**
Hands- on session	**8**	**3.5**	**0.49**	**14.1**	**3-4**
Discussion and summary	**8**	**2.9**	**0.35**	**12.2**	**2-3**
Report of workshop results	**7**	**3.1**	**0.58**	**18.4**	**2-4**
All scores		**3.3**	**0.70**	**21.3**	**2-5**

Mean values from the evaluations indicated that more time needed to be spent on *review of ageing procedures* (mean =3.8), *terminology and convention* (3.5), *review of ageing and validation studies* (3.5) and *hands-on session* (3.5). Mean values for other agenda items were near 3 (=just right). Overall, the workshop would have benefited by adding a half day or full day. The highest variation in responses occurred in review of winter flounder biology (CV=27.1) and review of regional biology (CV=26.8). The variation probably reflects the diverse experience of participants.

Overall, I thought the workshop met its terms of reference, especially given its multiple objectives. Ultimately, the success of the workshop will only be discernible if the report's recommendations, especially the development of reference collections, are implemented. Proclaiming consistency now and forever is one thing; providing objective and quantified evidence that consistency exists now and in the future is quite another. Implementing the workshop's recommendations should not be difficult, but they will require agencies to assign a priority to establishing the reference collection and implementing evaluation protocols as a routine part of their ageing program. These recommendations should dramatically reduce the probability of an ageing controversy arising in the future because consistency among readers will be constantly monitored and evaluated. If problems arise, they can be corrected without contaminating years of data.

APPENDIX IV Participants in the ASMFC Winter Flounder Ageing Workshop

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APPENDIX V Stock Areas for Winter Flounder in U.S. Waters

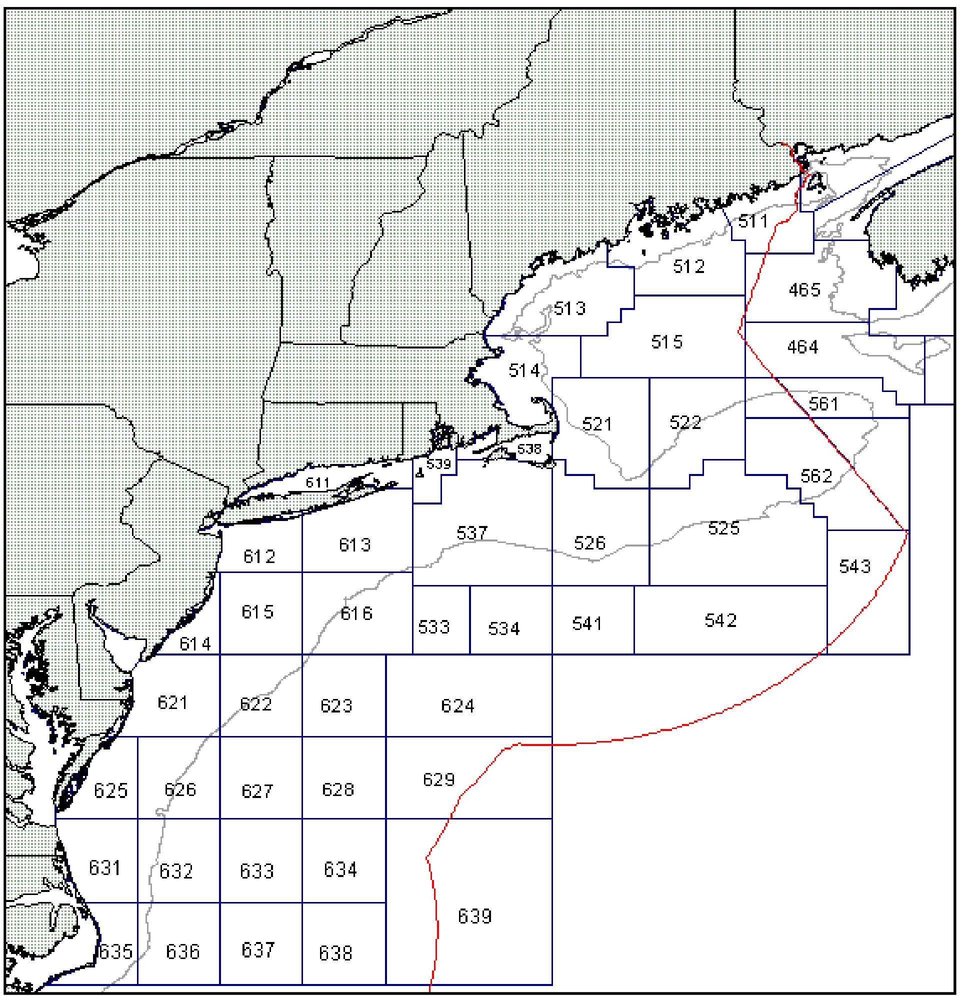
The following stock areas are currently used for assessment and management of winter flounder in U.S. waters:

Gulf of Maine [NAFO Div. 5Y, U.S. Statistical Reporting Areas (SAR) 511-515]

Southern New England-Middle Atlantic (NAFO Div. 5Zw and Subarea 6, U.S. SAR 521, 526, 537-539, 611-636)

Georges Bank (NAFO Div. 5Ze, U.S. SAR 522, 525, 561, 562)

Statistical areas used in stock definitions for winter flounder in United States waters. From the Northeast Fisheries Science Center.



APPENDIX VI Summary of winter flounder age validation study (Haas and Recksiek 1995)

Between October 1987 and December 1988, 732 winter flounder, 91-280 mm total length, were collected during biweekly sampling from Narragansett Bay, Rhode Island. Left sagittal otoliths from these fish were embedded in epoxy resin, and transverse sections through the foci were prepared. Objectives of the study were to document relationship between fish length and otolith section dimensions, verify ages of Narragansett Bay winter flounder using marginal increment analysis of sectioned otoliths, and compare ages determined from sectioned otoliths to those from whole otoliths and scales. With hyaline zones considered as annual increments, ages ranged from 1 to 11 for 608 winter flounder; 97% of the fish were younger than age 5. The anteroposterior otolith diameter was determined by linear regression analyses to be the best ($r^2 = 0.90$) of six “radial” axes for increment measurements. Marginal increment analyses for ages 1-4 showed that the increments, each composed of one opaque and one hyaline zone, are deposited annually, which clearly verified sectioned otolith ages for age-2 and age-3 fish. Opaque edges were prevalent in May, June, and July. Sectioned otoliths from winter flounder can provide clear increments and measurable increment widths through age 11. Two individuals read 369 sectioned otoliths and 116 whole otoliths; one individual read 155 scales twice. Precision between readers and aging methods was relatively high (average percent error, 1.5-4.5). Comparison of ages from scales and whole and sectioned otoliths from 154 fish showed no significant difference ($P \leq 0.05$). Conclusions were that 1) increment deposition occurs annually in winter flounder otoliths, providing a reliable means of age determination, at least through age four; 2) the sectioning method was verified through age four and is probably accurate for older ages; 3) sectioned otoliths from winter flounder can provide definite increments and measurable increment widths through age 11; and 4) for aging older fish, sectioned otoliths provide more accurate ages than whole otoliths or scales.

Literature cited

Haas, R.E. and C.W. Recksiek. 1995. Age verification of winter flounder in Narragansett Bay. Trans. Am. Fish. Soc. 124(1): 103-111.

APPENDIX VII   Glossary of Terms

(Taken from Penttila and Dery 1988)

Age determination notation

5 Five annuli counted (only one clear interpretation)

5(6) Probably five, but possibly 6 annuli (moderately difficult to age, two interpretations possible)

5? Five annuli is the best estimate (difficult to age, more than two interpretations possible)

5+ Five annuli counted with an additional seasonal growth increment.

Annulus - Any zone which forms once each year, usually the "winter" growth zone which marks the end of a year of growth.

Check - Zone of slow "winter" type growth that is not a true annulus. Such rings are distinguished by the width of the zone relative to annuli, and incomplete formation or poor definition. Checks may also be differentiated from annuli on some scales by differences in platelet shape.

Circulus - A concentric ridge formed on a scale by the periodic addition of material to the edge of the basal plate. The circuli on scales may be continuous or segmented by the scale radii, in which case the individual segments are termed platelets. Circuli are formed only on the outer surface of the scale; the inner surface is smooth

Collum - An interruption in the sulcus acusticus that marks the location of the nucleus

Crystallized otolith -An otolith displaying inadequate calcification. An age determination is not possible because of missing annuli.

Ctenoid scale - Type of scale having ctenii, or spine-like projections resembling the teeth of a comb, on its posterior edge.

"Cutting over" - (crossing over," erosion marks) disruption of the circulus pattern on scales from erosion of the edge results in circuli formed after erosion that appear to intersect or "cross-over" others that had been formed earlier. If scale edge erosion is an annual event, the "cutting-over" marks may be used to detect annuli.

Edge - Outer periphery of the age structure.

Edge type - Summer/winter or opaque/hyaline deposition occurring on the outer edge of the age structure representing the most recent growth.

End of annulus - Outermost edge of a winter growth zone designated as an annulus.

False annulus - Sometimes used synonymously with "check," refers to a zone of slow growth that is not counted as an annulus; also, a characteristic check ring on scales or otoliths which occurs before the first annulus and fairly close to the focus (scales) or nucleus (otoliths).

Focus - Center or origin of a scale.

Hyaline Zone - that allows the passage of light (also referred to as translucent). On otoliths, the "hyaline" zone is composed primarily of organic material (otolin) with a reduced amount of inorganic material in the form of short, thin calcium aragonite needles. With transmitted light, hyaline zones appear bright; with reflected light, they appear dark. "Winter" zones are normally composed of hyaline material.

Nucleus - Central portion of an otolith; sometimes called the core, kernel, or primordium.

Opaque - Zone that inhibits the passage of light. On otoliths, the "opaque" zone is composed primarily of inorganic calcium aragonite needles, which are long and thick relative to those formed in hyaline zones. With transmitted light, opaque zones appear dark; with reflected light, they appear bright. "Summer" zones are normally composed of opaque material.

Otolith teminology for age determinations (refer to Figures 3 - 6 for image showing general anatomy of otolith)

Platelets - Individual segments of a circulus on some types of scales, which are separated by the scale radii.

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

Sagittae - Largest of three pairs of otoliths located in the sacculus of the inner ear of a fish; referred to simply as "otoliths".

Settling check - Characteristic check ring on some marine groundfish otoliths. It occurs just outside the nucleus and is believed to form when the fish first become benthic in habit.

Shifted otolith - Otolith which has moved in the sacculus; recognized by additional growth occurring along a different axis from previous growth. Annuli may thus be present only on certain parts of a shifted otolith, and absent on other parts. Shifting often occurs in conjunction with crystallization of an otolith.

Split - Discontinuity in an annular zone, analogous to a "check." This causes the annulus to appear as two or more closely spaced "winter" zones.

Sulcus acusticus - (also referred to simply as "sulcus") Longitudinal groove extending down the convex surface of an otolith.

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