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Age determination, validation and growth of Grand Bank yellowtail flounder (*Limanda ferruginea*)

Karen S. Dwyer, Stephen J. Walsh, and Steven E. Campana

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Yellowtail flounder (*Limanda ferruginea*) (Storer, 1839) on the Grand Bank off Newfoundland were traditionally aged using surface-read whole otoliths. Age determination of otoliths from recaptures of fish tagged in the early 1990s indicated that the traditional ageing technique was underestimating the ages of yellowtail flounder when compared with the time at liberty. Age comparisons between whole and thin-sectioned otoliths showed agreement in age readings up to 7 years; thereafter whole otoliths tended to give much lower ages than those estimated by thin sections. Length–frequency analysis of pelagic and demersal juveniles, captive rearing of juveniles and marginal increment analysis all corroborated age determination based on thin sections. Tag-recaptures and bomb radiocarbon assays validated age interpretations based on thin sections in young and old yellowtail flounder, respectively. Ages were validated up to 25 years for females and 21 years for males. However, because of increased narrowing of annuli in thin-sectioned otoliths from old fish, even thin sections may underestimate the true age of the fish. von Bertalanffy growth curve parameters (combined sexes) were $L_\infty = 55.6$ cm total length, $K = 0.16$ and $t_0 = -0.003$. These results challenge the conventional view that yellowtail flounder on the Grand Banks are a relatively fast growing, short-lived species.

Keywords: age determination, Grand Bank, growth, validation methods, yellowtail flounder.

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K. S. Dwyer, and S. J. Walsh: Northwest Atlantic Fisheries Centre, Science Branch, Department of Fisheries and Oceans, PO Box 5667, St. John's, Newfoundland A1C 5X1, Canada. S. E. Campana: Marine Fish Division, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia B2Y 4A2, Canada. Correspondence to K. S. Dwyer.

Introduction

Yellowtail flounder, *Limanda ferruginea* (Storer, 1839), is a small-mouthed right-eyed pleuronectid found only in the Northwest Atlantic. Its distribution extends from the Strait of Belle Isle, off northern Newfoundland, south along the eastern seaboard of the United States to Chesapeake Bay, including the Gulf of St. Lawrence (Scott and Scott, 1988). The Grand Bank represents the northernmost limit of commercial concentrations of this species (Walsh, 1992) where a commercial fishery has operated since the 1960s (Pitt, 1970). Commercial concentrations are also found south on the Scotian Shelf, Georges Bank, and off Cape Cod (Walsh and Burnett, 2001).

Traditionally, yellowtail flounder have been aged using either scales or whole otoliths. Royce *et al.* (1959) concluded that the ages of the southern New England stocks of yellowtail flounder estimated from scales were accurate based on evidence such as edge type and length-frequency analysis. Scott (1947) concluded that whole otoliths were better than scales for age determination for Scotian Shelf yellowtail flounder; however, he did not give any confirmation of the accuracy of his method. Pitt (1974) asserted that Scott's (1947) study validated the use of whole otoliths for age determination in Grand Bank yellowtail flounder. Based on surface reading of whole otoliths, Pitt (1974) assumed that Grand Bank yellowtail flounder was a short-lived, fast-growing species that matured at age 5 (males) and 6 (females) and lived until age 13. He further concluded that the growth rates in Grand Bank yellowtail flounder, although slower than those found in New England stocks farther south, were similar to the Scotian Shelf stock. However, tag returns from a 1990–1993 Petersen disc tagging experiment (Morgan and Walsh, 1999) challenged Pitt's (1974) view of the age and growth dynamics of Grand Bank yellowtail flounder. Using recapture information on length and ages from otoliths returned with the tags, the calculated growth rate was found to be much slower than expected, while the time at liberty indicated that yellowtail flounder were living longer than the current age reading

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was indicating (Walsh and Morgan, 1999a). Such findings could have serious repercussions to the assessment of the resource and the understanding of the population dynamics of this species.

Accurate age determination of commercial fish is vital to successful fisheries management. Large-scale changes in age composition and parameters such as growth, mortality, maturity, recruitment and estimates of longevity are important indicators of the health of the stock. Errors in age-based assessment of growth and mortality rates and other life history parameters can lead to errors in the management of the fishery (Beamish and McFarlane, 1983). Typically, in marine fish, it is assumed that growth increments are laid down annually on hard parts, such as scales or otoliths. However, without age validation studies, some ageing methods may not provide the true age of the fish, nor certainty that an "annulus" is formed each year (see Campana, 2001 for a review). Therefore, age validation should be a priority in the ageing of commercial species (Beamish and McFarlane, 1983; Campana, 2001).

Although Grand Bank yellowtail flounder have been aged using whole otoliths since 1949, there have been no studies that corroborate or validate absolute age or periodicity of annulus formation. In this paper, we compare the traditional method of age determination of yellowtail flounder using whole otoliths with thin-sectioned otoliths from the same fish. We then use a variety of age corroboration and validation methods, such as length-frequency, identification of the first annulus, marginal increment analysis, tag–recapture analysis and bomb radiocarbon assays to assess the accuracy of these age determination methods and examine growth in yellowtail flounder. We conclude by discussing the consequences of this new information on the life history of yellowtail flounder.

Materials and methods

Age determination: comparison of methods

The traditional method of age determination of yellowtail flounder from the Grand Bank stock carried out by the Northwest Atlantic Fisheries Centre (NAFC), Department of Fisheries and Oceans' Newfoundland region, was based on surface reading of whole otoliths. When necessary, surface grinding of older fish otoliths, using a rotary grinding wheel, was attempted in an effort to make the outer rings more visible. A comparison of this method of age determination with the broadly accepted thin-sectioning method was investigated by comparing ages estimated from whole and thin-sectioned otoliths from the same fish. Both sagittal otoliths were removed from 370 randomly selected yellowtail flounder from the Grand Bank, Northwest Atlantic Fisheries Organization (NAFO) Divisions 3LNO, during annual research bottom trawl surveys in 1999 and 2000 and stored dry. All otoliths were aged twice by the two readers, without prior information on length, sex or time of capture. In this comparison, the readings were done in a random order. An earlier experiment indicated that surface grinding did not improve the visibility of rings on older otoliths, and did not change the age estimate; therefore otoliths were not ground (Whalen *et al.*, 2000). After the whole otoliths were read, both otoliths were sectioned, so that choice of otolith was not confounded with the method used. Otoliths were embedded in wax and an Isomet low-speed saw was used to cut an approximately 0.3–0.8 mm thick transverse section through the primordium, along the dorsolateral plane of the otolith. In later analyses, we obtained thinner sections as the method improved. Because of the brittle nature of smaller otoliths, otoliths from fish less than 30 cm were cut transversely and each half of the otolith was examined.

Whole otoliths were placed in a black chamber containing ethyl alcohol and examined using a Nikon dissecting microscope under reflected light at 8–20× magnification according to the traditional methods of examination at NAFC. The number of winter-hyaline (translucent) zones (annuli) was recorded. Sectioned otoliths were also examined in alcohol using magnifications of 25–40× with reflected light.

The bias and precision of annulus counts were compared among readers, methods and left/right otolith, using paired t-tests and age bias plots (Campana *et al.*, 1995; Campana, 2001). For each paired comparison, a coefficient of variation (CV) was also used to measure precision together with a paired t-test to compare differences statistically.

Age corroboration and validation methods

Length-frequency analysis

Length frequencies of pelagic juvenile yellowtail flounder were examined from annual NAFC pelagic 0-group surveys of the Grand Bank during late August and early September, 1994–1999 (J. Anderson, NAFC: unpubl. data) using an *International Young Gadoid Pelagic Trawl* (*IGYPT*). In addition, historical data from pelagic 0-group surveys conducted with a small BIONESS multiple opening and closing net during each September from 1986 to 1988 were examined (Frank *et al.*, 1992; with permission from J. Carscadden, NAFC). Mean lengths were produced by averaging the samples present for each year.

The Petersen method (Petersen, 1892) was used to corroborate ages of younger fish by examining modal frequencies in length distribution plots from annual NAFC juvenile groundfish surveys. The method produces an approximate size for distinct modes, which are believed to represent the fast growing early ages. The NAFC surveys used a small mesh *Yankee 41* shrimp trawl, and were conducted in late August and early September surveys from 1985 to 1994. The length frequency of each survey catch was plotted and the modal length for each peak estimated by adding the visible upper limit and lower limit and dividing this sum by two.

Length-at-age values derived for length frequencies (Table 1) are estimated using RMix (Du, 1999). This

Table 1. Length-at-age for each ageing method (cm). Values represent means ± standard deviation (except length-frequency values, which are mean ± standard error from RMix) with sample size in parentheses.

Age	Length–frequency	Section ♂	Section ♀	Tag–recapture ♂	Tag–recapture ♀	Bomb radiocarbon ♂	Bomb radiocarbon ♀
0	3.0 ± 0.1	–	–	–	–	–	–
1	6.6 ± 0.0	12.0 ± 3.8 (5)	9.8 ± 3.2 (8)	–	–	–	–
2	12.4 ± 0.1	14.1 ± 2.7 (23)	15.0 ± 3.5 (23)	–	–	–	–
3	20.8 ± 0.1	19.5 ± 3.3 (12)	20.5 ± 2.7 (13)	–	–	–	–
4	–	24 ± 3.1 (12)	24.5 ± 3.0 (10)	–	–	–	–
5	–	28.8 ± 5.6 (12)	27.2 ± 2.2 (12)	–	–	–	–
6	–	33.7 ± 4.9 (14)	35.0 ± 4.7 (19)	–	–	–	–
7	–	36.4 ± 3.8 (11)	38.6 ± 5.1 (20)	33.3 ± 1.3 (3)	31.5 ± 1.0 (2)	–	–
8	–	38.0 ± 4.3 (10)	42.8 ± 4.0 (18)	36.0 ± 1.1 (3)	–	–	–
9	–	42.6 ± 4.0 (17)	44.2 ± 4.9 (12)	33.0 (1)	39.0 ± 9.8 (2)	–	–
10	–	43.5 ± 3.9 (24)	45.6 ± 3.8 (28)	36.5 ± 2.9 (2)	43.0 (1)	–	–
11	–	44.6 ± 3.0 (7)	48.3 ± 3.0 (15)	35.0 ± 3.9 (2)	39.7 ± 2.4 (3)	–	–
12	–	43.7 ± 3.3 (9)	48.1 ± 4.0 (11)	36.3 ± 3.8 (4)	41.0 (1)	–	–
13	–	42.5 ± 4.2 (4)	47.8 ± 4.6 (11)	36.7 ± 0.7 (3)	41.0 ± 2.3 (3)	–	52.0 ± 1.4 (2)
14	–	46.5 ± 3.5 (2)	49.0 ± 8.5 (2)	42.0 (1)	–	47.0 (1)	51.8 ± 1.7 (4)
15	–	–	50.8 ± 4.2 (5)	–	–	45.0 (1)	51.0 (1)
16	–	–	–	–	–	45.0 ± 0.0 (2)	51.3 ± 0.5 (4)
17	–	–	–	–	–	–	50.0 ± 3.5 (3)
18	–	–	–	–	–	49.0 (1)	53.5 ± 2.1 (2)
19	–	–	–	–	–	–	53.8 ± 1.2 (4)
20	–	–	–	–	–	–	53.0 ± 0.0 (4)
21	–	–	–	–	–	49.0 (1)	51.0 (1)
22	–	–	–	–	–	–	54.0 (1)
23	–	–	–	–	–	–	50.0 (1)
24	–	–	–	–	–	–	–
25	–	–	–	–	–	–	56.0 (1)

program fits mixture distributions to modal data by the method of maximum likelihood. This enables us to obtain a mean ± standard error for the 1987 length-frequency for males.

Determining the first annulus

To validate the first growth increment, measurements of the diameter of young-of-year otoliths at the time of annulus formation were taken (Campana, 2001). Otoliths from three fish (<6 cm) captured in the spring surveys of the Grand Bank using a Campelen 1800 shrimp trawl, presumably when the first annulus should be laid down, were available. The diameter of the sectioned left otolith was measured using an Optimas 6.2 image analysis system. The diameter (mm) was measured across the widest part of the otolith, along the same axis used to estimate the age of the fish. Because so few young-of-year fish were available, fish between 6 and 8 cm ($n = 8$), which had just laid down their second annulus in the spring survey, were also used in the measuring of the first annulus.

In addition, we used mean young-of-year fish length (calculated by RMix for the 0-group male fish in November 1987) in the season of annulus formation (since there is little growth over winter, the length of fish in the season of annulus formation should be the same as in November) and the regression of otolith diameter on fish length to predict the expected diameter of the first annulus (Campana, 2001).

Captive rearing of yellowtail flounder from hatch

Yellowtail flounder were reared from eggs hatched at Memorial University of Newfoundland's Ocean Sciences Centre in Logy Bay. They were kept in a flow-through facility, under natural photoperiod and fed a commercial diet. For the first year of life, fish were kept under a temperature regime of 7–13°C. The 1+ fish were reared under a temperature regime of 4–11.7°C and the 2+ fish held at a separate facility under a more extreme temperature regime (1–15°C). Twelve age 1 and 12 age 2 juveniles were killed and the otoliths removed for examination. Both whole otoliths and thin sections were used to estimate ages, and compared to the actual ages of the fish. The age reader had no prior knowledge of length or age.

Marginal increment analysis

Otoliths ($n = 135$) collected from yellowtail flounder captured during seasonal groundfish surveys of the Grand Bank throughout the years 1985–1986 were used in the marginal increment analysis. A low-speed Isomet saw with double blades was used to remove a 0.5-mm transverse section of the left otolith. These otoliths were immersed in alcohol and aged under reflected light twice, by the same age reader, to derive an acceptable level of precision. The reader had no prior knowledge of length, sex or time of capture.

The marginal increment was measured as extra growth expressed as a proportion of the previous year's growth. An Optimas® 6.2 image analysis system was used to record the two measurements at 40× magnification. Annuli were counted along the dorsal edge of the otolith and followed along to the *sulcus acusticus* (defined as the longitudinal groove extending down the convex surface of an otolith) where possible. Fish less than and equal to 30 cm (aged 3–7 years) were used to represent immature and/or actively growing fish, and were pooled into one group because of low sample sizes. In addition, because fish were not collected for each month, months were pooled into seasons, from winter 1984 to winter 1985.

Tag–recapture analysis

In the early 1990s, 9522 yellowtail flounder, ranging in size from 15 to 35 cm total length, were captured, tagged and released on the southern Grand Bank (Morgan and Walsh, 1999). Otoliths from 37 fish recaptured mainly by the commercial fishery, and some research vessels, from 1993 to 2000, were used in the tag-recapture analysis. Because these fish were either juveniles or young adults when tagged, age-at-release could be estimated reasonably accurately using back-calculation equations. As a result, these recaptured fish could be considered close to known age. Otolith size at the age of release was estimated using a re-arrangement of the Fraser–Lee equation (Campana, 1990):

$$L_a = d + (L_c - d)O_c^{-1}O_a \tag{1}$$

where L_a is fish length at some previous age (a), O_a the measured size (radius) of the otolith at age a and d the intercept of the fish length–otolith measurement regression, using fish only up to 44 cm in length. L_c and O_c are fish length and otolith radius, respectively, at capture (c). Equation (1) was then re-arranged to solve for O_a as follows:

$$O_a = ((L_a - d) \times (O_c))/(L_c - d) \tag{2}$$

The relationship between fish length and otolith radius was determined using tagging samples and juveniles in order to find d, the y-intercept. Thin sections of the left otolith were examined under reflected light with a Nikon® SMZ-10A stereo-microscope. Magnification depended on the size of section and varied from 7.5 to 40×. Where necessary, an image of the section was viewed with a Sony® video camera (STP-M314) and digitally enhanced in Adobe Photoshop 4.0. Annulus age was based on the mean of repeated counts. The radius of each section was then measured with Optimas® 6.2 along the reading axis, from the dorsal tip to the outer edge of the otolith. Once Equation (2) was solved for O_a, the number of annuli distal to O_a was compared to the number of years at liberty.

Bomb radiocarbon assays

Thirty-four pairs of otoliths, from 6 males and 28 females, were selected from archived material collected from research surveys carried out on the Grand Bank between 1972 and 1988. The largest and seemingly the oldest fish, which may have hatched in the 1950s and 1960s, were selected since these are the year classes most suited to bomb radiocarbon dating. Annulus age was estimated from one or both sagittal otoliths of each pair using the criteria described earlier. Thin sections (~1 mm thick) of each otolith were prepared by sectioning transversely through the core. The radius of the presumed first annulus was confirmed through measurements of the dimensions of intact sagittae collected from young of the year yellowtail flounder, which indicated that the first annulus of a transverse section should be approximately 0.73 × 0.30 mm wide (see previous section). After ageing, the remaining pieces of each otolith were stored dry in paper envelopes in preparation for ^{14}C assay.

To isolate otolith material for bomb radiocarbon assay, otolith cores corresponding to the first 2 years of growth (medial to the second translucent zone) were extracted from each thin section. Cores were isolated with a precision, high speed, rotary handset (Gesswein Power Hand 2X) using diamond cutting bits and steel burrs. Since individual core weights were insufficient for assay, cores were isolated from each otolith of the pair and pooled. Most samples were also pooled with one other fish of the same age and hatch date (± 1 year), so as to bring total sample weight to at least 7 mg. All core material was then decontaminated, wrapped in aluminium foil and submitted for ^{14}C assay by accelerator mass spectrometry (AMS) (as described by Campana, 1999). AMS assays also provided $\delta^{13}C$ values, which were used to correct for isotopic fractionation effects. Radiocarbon values were subsequently reported as $\Delta^{14}C$, which is the permil (‰) deviation of the sample from the radiocarbon concentration of 19th-century wood, corrected for sample decay prior to 1950 according to methods outlined by Stuiver and Polach (1977).

A reference $\Delta^{14}C$ curve was prepared through radiocarbon assay of 1–3 year old haddock (*Melanogrammus aeglefinus*) and redfish (*Sebastes* spp.) otolith cores of known age. Both the redfish and haddock cores were prepared and assayed in a similar fashion (Campana, 1997). The period of $\Delta^{14}C$ increase was virtually identical in both species, and synchronous with that of corals and bivalves growing at the time; therefore the reference curve provides a known and dated $\Delta^{14}C$ series against which the yellowtail flounder core assays can be compared. Uncertainty around the reference line is no more than 2 years between 1957 and 1965.

Growth curves

von Bertalanffy growth curves, based on length-at-age from all age readings, including those from the earliest length-frequency modes, thin-sectioning, tag–recapture analysis and bomb radiocarbon assays, were fitted separately for males and females by non-linear regression:

$$l_t = L_\infty(1 - e^{-K(t-t_0)}) \quad (3)$$

where l_t is the length-at-age t, K a growth rate parameter ($year^{-1}$), L_∞ the mean theoretical maximum total length and t_0 the theoretical age at zero length. Curves were fitted to the length-at-age data for each sex using R script (Ihaka and Gentleman, 1996).

Results

Age determination: comparison of methods

Distinct growth zones, presumed to be annuli, were observed for the first 5–7 years in both whole and thin-sectioned otoliths. Subsequent annuli were more difficult to see, especially in whole otoliths. Spacing between subsequent annuli decreased with age, and narrowed substantially in the older individuals (Figure 1). The left or symmetrical otolith was found to give the most consistent readings. Ager 1 tended to underestimate ages of the right otolith in older fish (CV = 15.4%; $p < 0.0001$), while there was no difference in ages estimated between left and right otolith by Ager 2 (CV = 10.1%; $p = 0.8077$).

Age bias plots (not shown) for whole and thin-sectioned otoliths showed no systematic bias between the two age readers for each method (whole otoliths between readers: CV = 8.3%, $p = 0.0949$; sectioned otoliths: CV = 11.5%, $p = 0.9151$). However, age bias plots of ages estimated from thin sections compared to ages estimated from whole otoliths revealed a large discrepancy in agreement between the two methods (Figure 2). Beginning at age 8, ages estimated from whole otoliths tended to be much lower than those estimated from thin sections. In the oldest fish, whole otoliths underestimated the age indicated in thin sections by about 50%. There was also a tendency to count

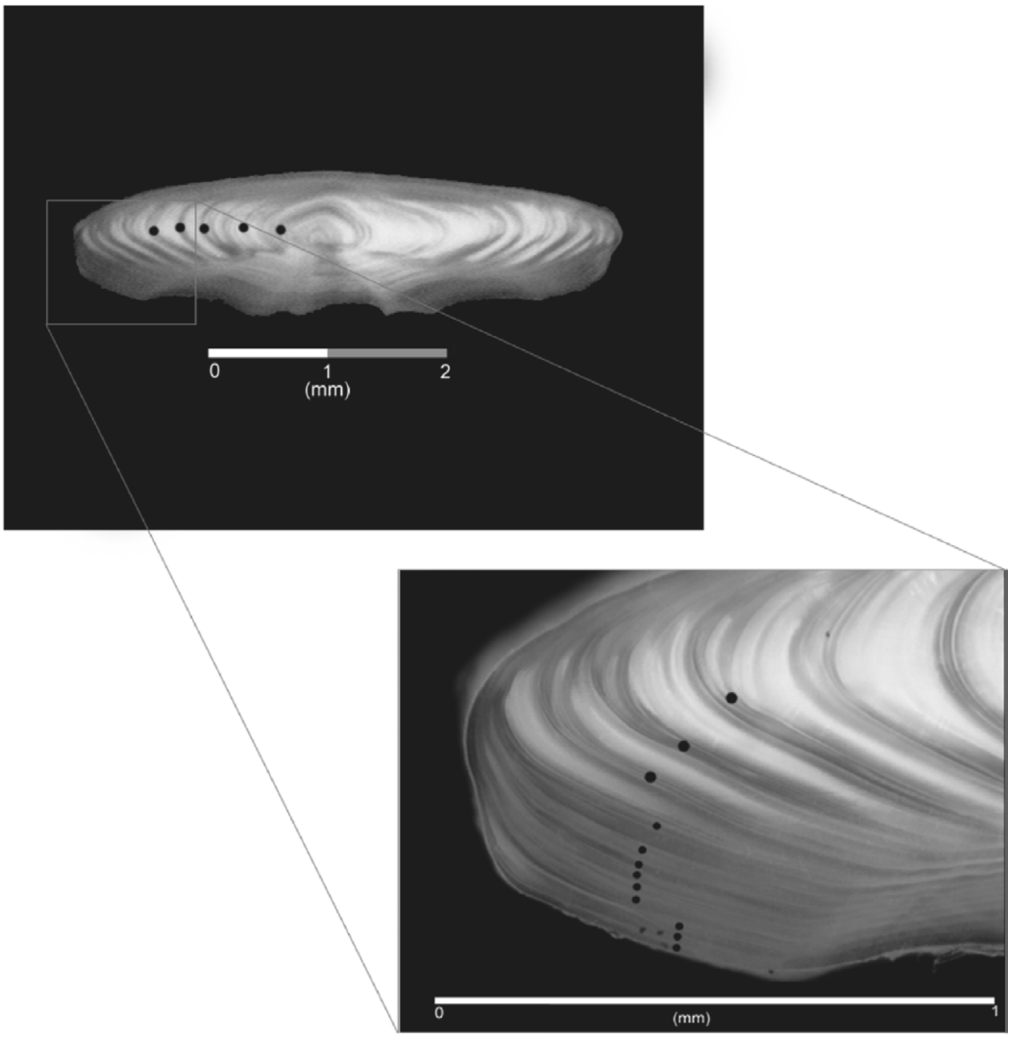

Figure 1. Transverse section of a yellowtail flounder otolith (46 cm female), revealing the annuli (black dots). Annuli formed before sexual maturation (top insert) look very different from those formed after sexual maturation (bottom insert).

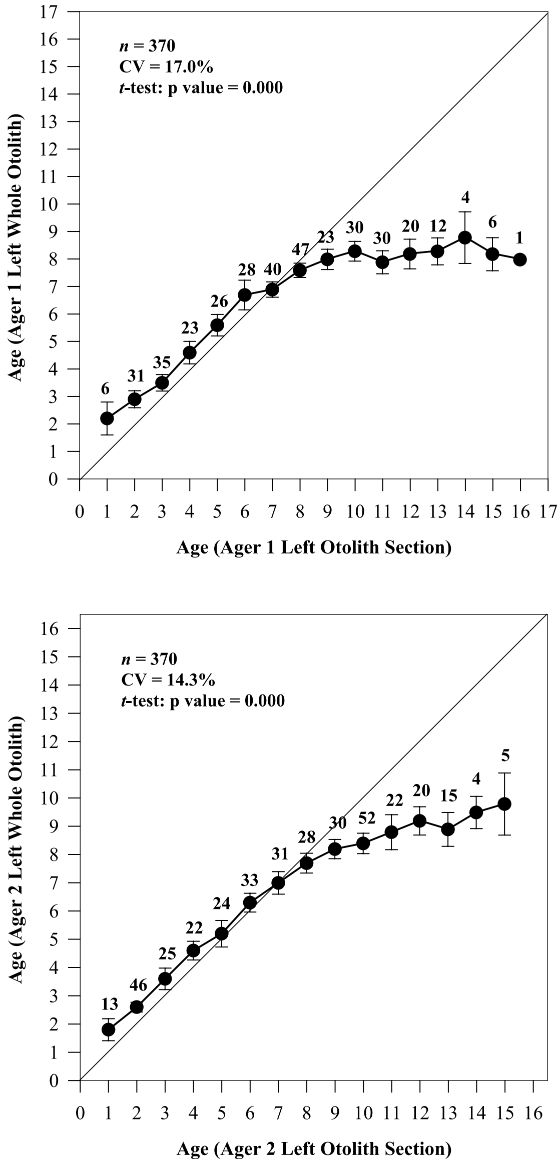

Figure 2. Age bias plot for Agers 1 and 2 ageing whole otoliths and sectioned otoliths. Each error bar represents the 95% confidence interval about the mean age assigned for one otolith for all fish assigned a given age for the second otolith. The 1 : 1 equivalence (solid line) is also indicated. Numbers plotted with symbols are the sample size at each age.

an extra annulus in whole otoliths due to a mis-interpretation of the first annulus, which was later confirmed in the validation of the first growth increment.

Age corroboration and validation

Length-frequency analysis

Length-frequency distributions of pelagic 0-group yellowtail flounder from the southern Grand Bank in September 1986–1988 had a length range of 4–34 (grand mean ± S.D. = 2.0 ± 0.7 cm, n = 682) (Figure 3). In the August 1994–1999 IGYPT surveys the 0-group ranged in size from 0.2 to 4.2 cm (mean ± S.D. = 1.4 ± 1.3 cm, n = 71), however, there was insufficient sample size for further analysis. Length-frequency distributions of yellowtail flounder from annual juvenile groundfish surveys of the Grand Bank carried out in August–September 1985–1994 showed up to three distinct modes in most years, i.e. 6.5–7.5, 12.5–17 and 18–26 cm (Figure 4). In 1986, 1987 and 1989, a fourth peak was detected at ~3 cm; this mode was larger (and therefore visible) in 1987 because the delay in timing of the survey to November allowed the gear to capture more demersal 0-group fish. Since the pelagic 0-group results showed a mean length of 2.6 cm in August of 1987 (Table 1, Figure 3) we assume that this 3 cm modal peak in the 1987 survey represents the newly settled 0-group demersal phase and the three other modal sizes above are ages 1–3. The strong 1985 year-class at age 1 in 1986 can be followed to about age 5 (Figure 4).

Determination of the first annulus

An examination of the otoliths from the annual spring bottom trawl surveys of the Grand Bank revealed only a few 1-year-old yellowtail flounder (<6 cm). The first annulus of thin-sections had an average diameter of 0.07 ± 0.01 cm (n = 3). The first annulus of age 2 fish (6–8 cm in length) from the spring surveys was also measured to increase sample size. Thus we would expect that the first (and hence all other) annulus was formed in the March–May period. The diameter of the first annulus of these 2-year-old fish ranged in size from 0.04 to 0.08 cm (mean ± S.D. = 0.06 ± 0.01 cm, n = 9) and its thickness ranged in size from 0.03 to 0.04 cm, whereas the second annulus had a diameter of 0.1 ± 0.01 cm. Although additional measurements of the diameter of the first annulus from otoliths of larger fish (>45 cm; n = 14) resulted in a larger mean diameter (0.07 ± 0.01 cm) than that found in 2-year-old fish, a t-test showed that the difference in size was not significant (p = 0.161).

To further validate the identity of the first annulus, the mode (3.0 cm) of the demersal 0-group males from the fall 1987 length frequency (estimated from RMix) was inserted into a fish length-otolith diameter regression to predict the annulus diameter (Figure 5). The predicted annulus diameter of 0.07 cm was comparable with the observed measurements derived above. Therefore we concluded that the first ring visible on the otolith was considered the first annulus. This method is discussed in Campana (2001) and has been used to validate the position of the first annulus in other species, such as haddock (Campana, 1997).

Captive rearing of yellowtail flounder from hatch

Periodicity of annulus formation was also observed by examining juveniles (n = 24) reared from fertilized eggs in

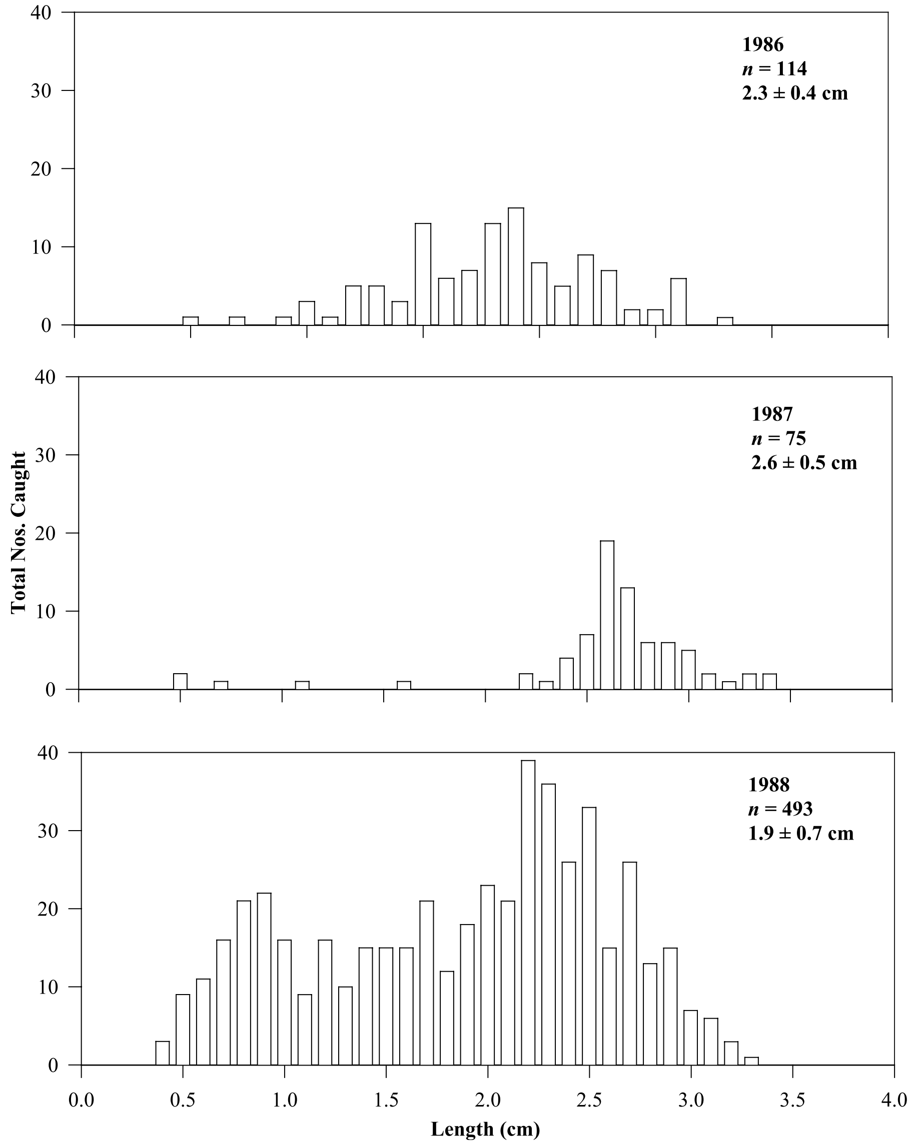

Figure 3. Length frequencies for pelagic yellowtail flounder larvae from surveys conducted on the southern Grand Banks during September for 1986–1988 (from Frank *et al.*, 1992). Values are mean ± standard deviation.

a laboratory. Samples of otoliths were taken 1 ($n = 12$) and 2 years ($n = 12$) after hatching in April. When compared, annulus counts matched the known age of the fish for the first and second years of life (Figure 6A,B). These results corroborate the view that the otolith annuli were being correctly interpreted.

Marginal increment analysis

Thin-sectioned otoliths of 3 to 7 year-old fish sampled during quarterly surveys from 1985 to 1986 showed an annual cycle in the monthly marginal increment (Figure 7). The increment decreased in the winter of 1984 to a low value of 0.21 in the spring and rose in the summer months to peak at 0.70 in the fall. The marginal increment fell back down to a similar low value in the winter of the second year. This cyclical pattern is consistent with the formation of one annulus per year. It appears as if the annulus (translucent zone) is laid down in March/April (spring).

Tag–recapture analysis

The size of the 32 recaptured fish used in the analysis ranged from 31 to 43 cm and the time-at-liberty ranged from 1.3 to 10.5 years. There was a significant relationship between fish length (cm) and otolith radius (mm) of recaptures with a y-intercept (d) of 0.4515, a slope of 0.0457 and an r^2 of 0.92. These data were then used to

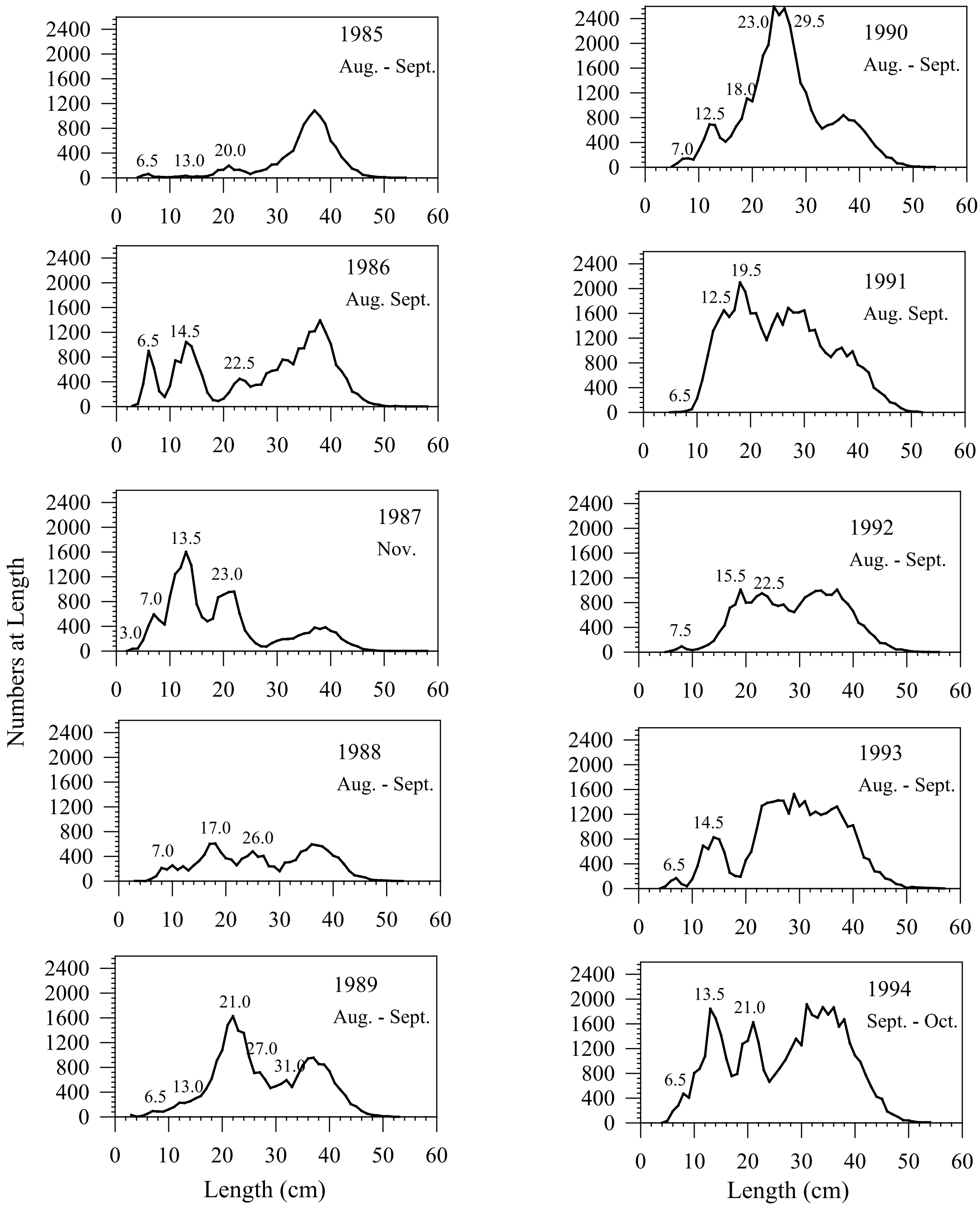

Figure 4. Length compositions (cm) of yellowtail flounder (both sexes) from annual juvenile groundfish surveys from 1985 to 1994. Time of year is indicated. Numbers are modal lengths as inspected visually (cm).

predict the age of the fish at time of release, and were subtracted from the total annulus count at recapture to estimate the number of annuli formed after tagging. Mean age at tagging was estimated to range between 1 and 7 years and age at recapture ranged from 7 to 14 years.

The age bias plot of number of annuli formed after tagging and the time at liberty of recaptures showed a departure from agreement for fish at liberty beyond age 8 years (CV = 13.5%; a paired t-test with p-value of 0.0485) (Figure 8). However, when the same test was carried out on fish that were at liberty less than 9 years, there was no significant difference ($p = 0.4610$) between time at liberty and number of annuli counted after O_a. This latter result validates the interpretation that one annulus is formed each year until at

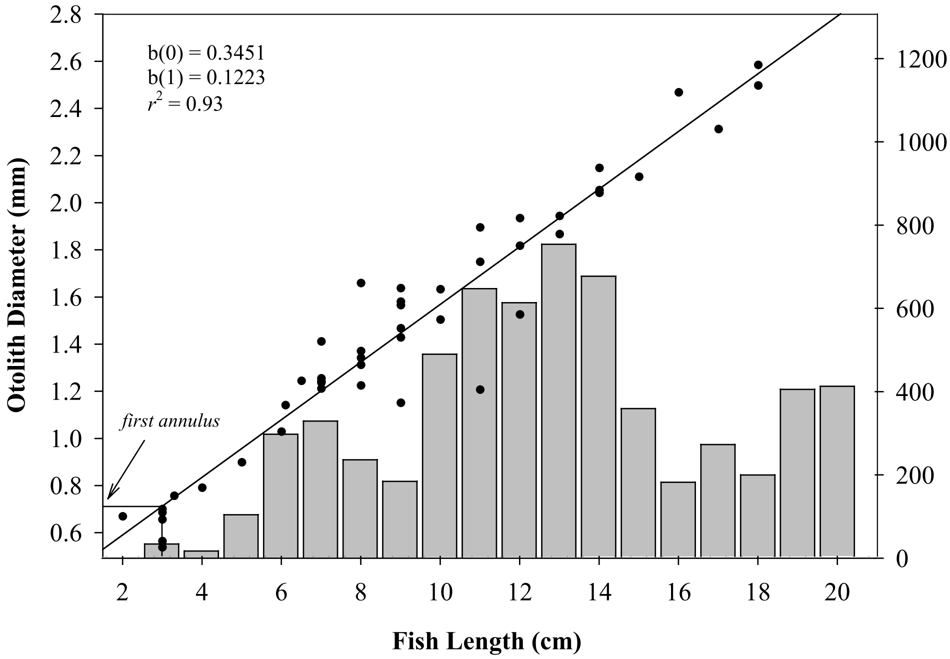

Figure 5. Validation of the identity of the first annulus. Using the estimated mean corresponding to the 0-group fish in a length-frequency plot (data from male fish in spring 1987 equivalent to 1 year olds), annulus diameter is predicted on the basis of a fish-length otolith-diameter regression (using samples across a number of years). This annulus diameter closely matches that of the first annulus of otoliths from fish measured in spring just after annulus formation.

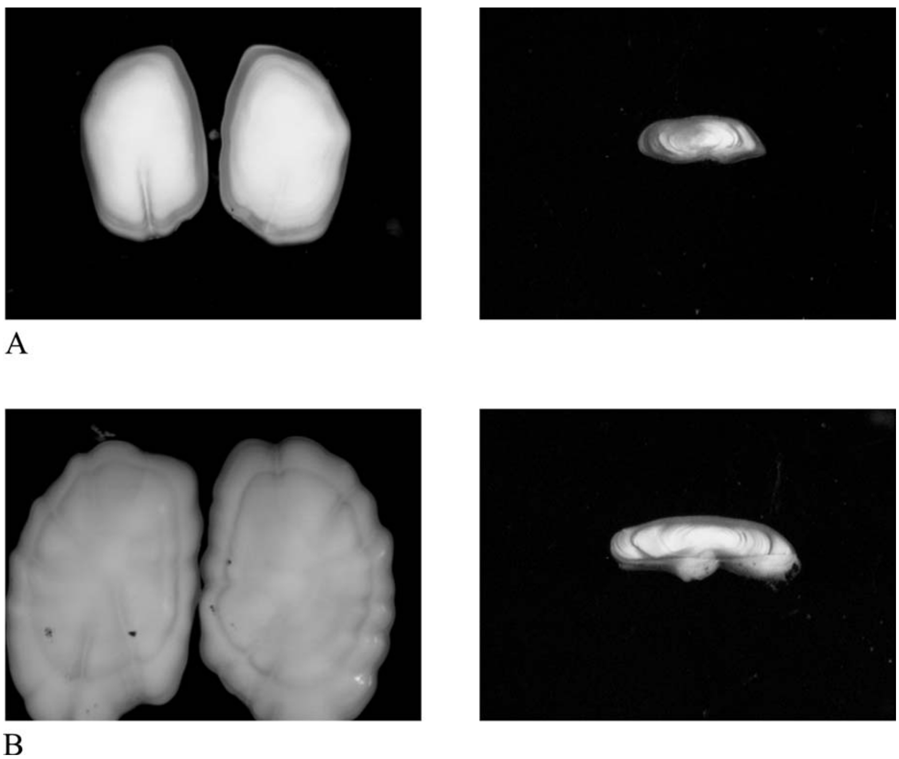

Figure 6. (A) Otoliths removed from 1+ yellowtail flounder reared in captivity. Fish were killed in April. (B) Otoliths removed from 2+ yellowtail flounder reared in captivity. Fish were killed in April.

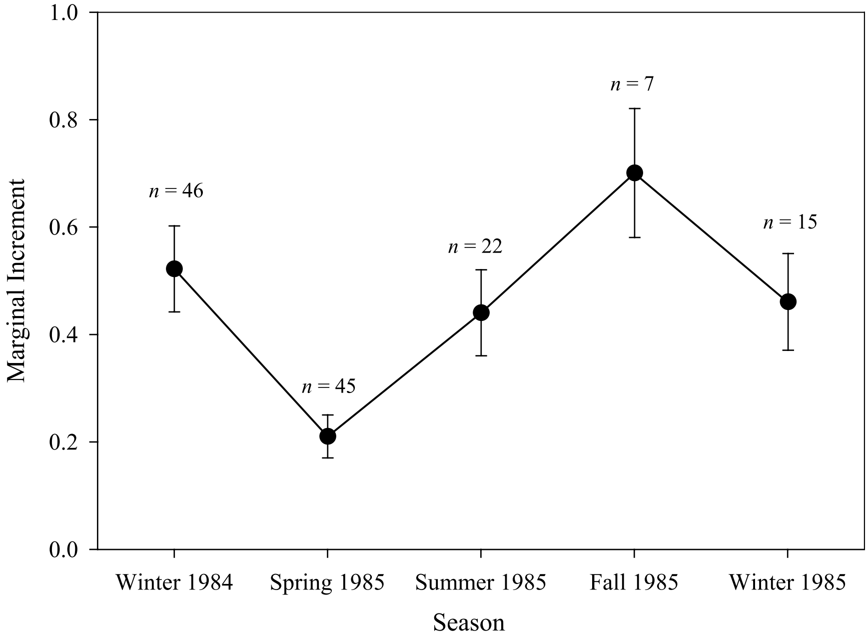

Figure 7. Monthly changes in mean marginal increment for sectioned otoliths of yellowtail flounder. Numbers indicate sample size.

least age 13. However, the departure from agreement with increasing years at liberty suggests that there is an underestimation of yellowtail flounder at older ages (>13 years) even with thin sections.

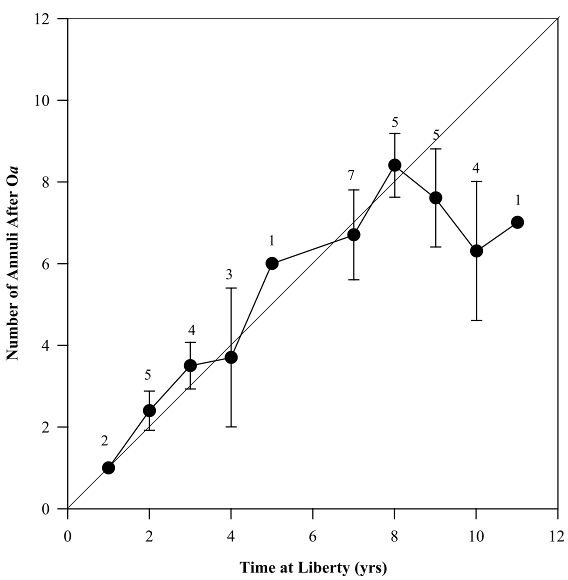

Figure 8. Age bias plot showing the difference between time at liberty for tag–recaptured yellowtail flounder and the number of annuli formed after tagging. Each error bar represents the 95% confidence interval of the number of annuli formed after tagging for a given time at liberty. The 1 : 1 equivalence (solid line) is also indicated. Numbers plotted with symbols are the sample size for each. O_a is the radius of the otolith at age a.

Bomb radiocarbon assays

The date of formation of the yellowtail flounder otolith cores was estimated in two ways: through age determination based on annulus counts of thin-sectioned otoliths, and through comparison of otolith core $\Delta^{14}C$ values with the values known to be present in the marine environment at the time. The period of increasing $\Delta^{14}C$ values in the otolith cores can be compared to the known-age and dated reference values to determine the date of formation. Where the annulus-based and $\Delta^{14}C$-based dates are in agreement, the annulus-based age interpretations must be (on average) correct.

$\Delta^{14}C$ values in the otolith cores varied between -80 and 54, similar to reported values of other marine carbonates formed in the 1950s and 1960s. The standard deviation of individual $\Delta^{14}C$ assays ranged between 4.7 and 5.3. There was no significant relationship between $\delta^{13}C$ and either presumed age or hatch date; mean $\delta^{13}C$ was -2.51 (s.e. $= 0.13$).

All of the otoliths aged for radiocarbon assays were from old fish: the range of annulus-based ages was 13–25 (Figure 9). Readings by a secondary reader were generally consistent, but the estimates were a few years older. If the annulus-based ages are assumed to be correct and are used to determine the year of core formation, a plot of $\Delta^{14}C$ against year of core formation shows the curve expected of all marine carbonates: low and relatively stable values prior to 1957, increasing sharply to an asymptote in the late 1960s (Figure 9). However, comparison with the accurately dated reference $\Delta^{14}C$ curve indicates that most of the annulus-based age determinations were underaged. The yellowtail flounder assay results are offset by an average of

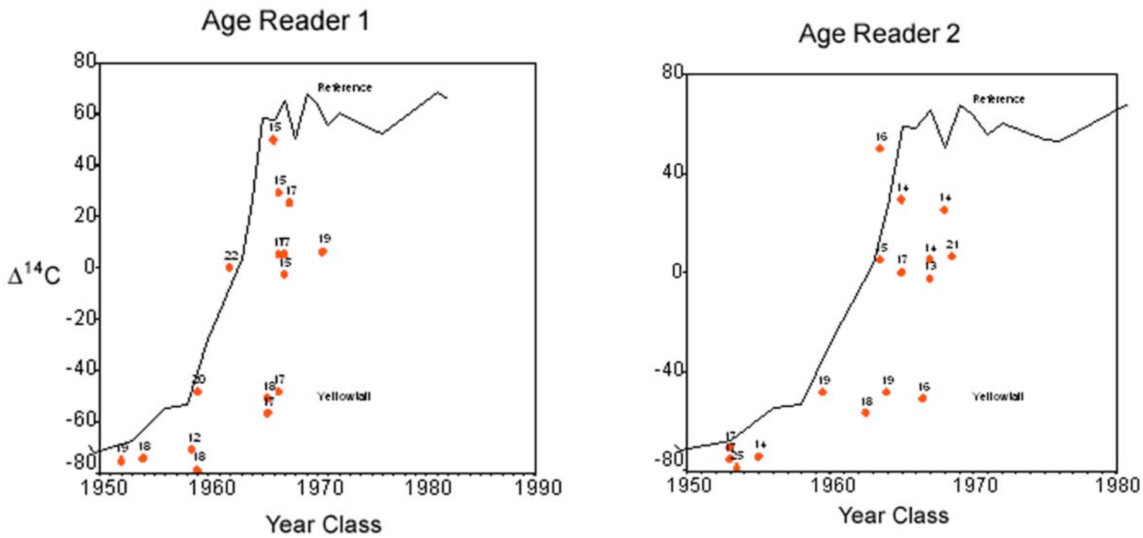

Figure 9. $\Delta^{14}C$ concentrations in the otoliths of old yellowtail flounder in relation to published $\Delta^{14}C$ chronologies for redfish (*Sebastes* spp.) and haddock (*Melanogrammus aeglefinus*) (solid reference line) (Campana, 1997). Numbers (and red dots) indicate individual age estimates.

about 5 years compared to the reference curve, indicating that the annulus counts were underaged (on average) by about 5 years.

Growth curves

A comparison of the growth curves for male and female yellowtail flounder, as well as combined sexes, is given in Figure 10. von Bertalanffy growth curves were fitted to all of the aggregated data from various sources (i.e. length frequency, sections, tagging and bomb radiocarbon dating otoliths). Males reached a maximum age of 21 years and a maximum size of 50 cm and females reached a maximum age of 25 years and a maximum size of 56 cm. The growth rates for both males and females were similar up to age 4 years but after that it appears that females grow faster than males (Figure 10). Predicted length-at-age from von Bertalanffy equations at age 8 for females was 40.8 cm and for males 48.6 cm, whereas at age 17, it was 52.6 cm for females and 48.9 cm for males. The von Bertalanffy growth equations were compared with other studies in Table 2.

Discussion

The traditional method of ageing whole otoliths from the Grand Bank yellowtail flounder stock has been shown to be unreliable for fish beyond age 7 years. There are more annuli visible in thin sections of older fish compared to that seen on the surface of whole otoliths and our results indicate that thin sections provide an accurate age estimate for all but the very oldest fish. The underageing of older fish using the whole otolith method occurs because yearly growth increments do not form equally on all parts of the otolith (Chilton and Beamish, 1982). This has been demonstrated in a number of species of fish, for example, Pacific ocean perch (*Sebastes alutus*) (Beamish, 1979), shortbelly rockfish (*Sebastes jordani*) (Pearson *et al.*, 1991) and other rockfish and flatfish species (in Chilton and Beamish, 1982). Age determination of Grand Bank yellowtail flounder using scales, whole otoliths and thin-sectioned otoliths gave similar results for fish up to age 7 years (Dwyer *et al.*, 2001). Dwyer *et al.* also reported that there were no differences in age determination obtained from baked thin sections and regularly prepared thin sections.

Age determination using cross sections of the otolith has been validated in a vast number of species. A number of different validation methods have been used, such as chemical marking, using oxytetracycline in English sole (*Parophyrs vetulus*) (MacLellan and Fargo, 1995), sablefish (*Anoplopoma fimbria*) (McFarlane and Beamish, 1995), red drum (*Sciaenops ocellatus*) (Murphy and Taylor, 1991) and yellowtail rockfish (*Sebastes flavidus*) (Leaman and Nagtegaal, 1987) to validate burnt cross sections of otoliths. In redfish (*Sebastes mentella*) (Campana *et al.*, 1990) and Pacific grenadier (*Coryphaenoides acrolepis*) (Andrews *et al.*, 1999), thin sectioning of otoliths was validated by the use of the ^{210}Pb:^{226}Ra ratio in the otolith core, and finally, age validation has been achieved in black drum (*Pogonias cromis*) (Campana and Jones, 1998), blue grenadier (*Macruronus novaezelandiae*) (Kalish *et al.*, 1997), haddock (Campana, 1997) and southern bluefin tuna (*Thunnus maccoyii*) (Kalish *et al.*, 1996) by using assays of bomb radiocarbon content. The only attempt at age validation in yellowtail flounder was carried out by Royce *et al.* (1959)

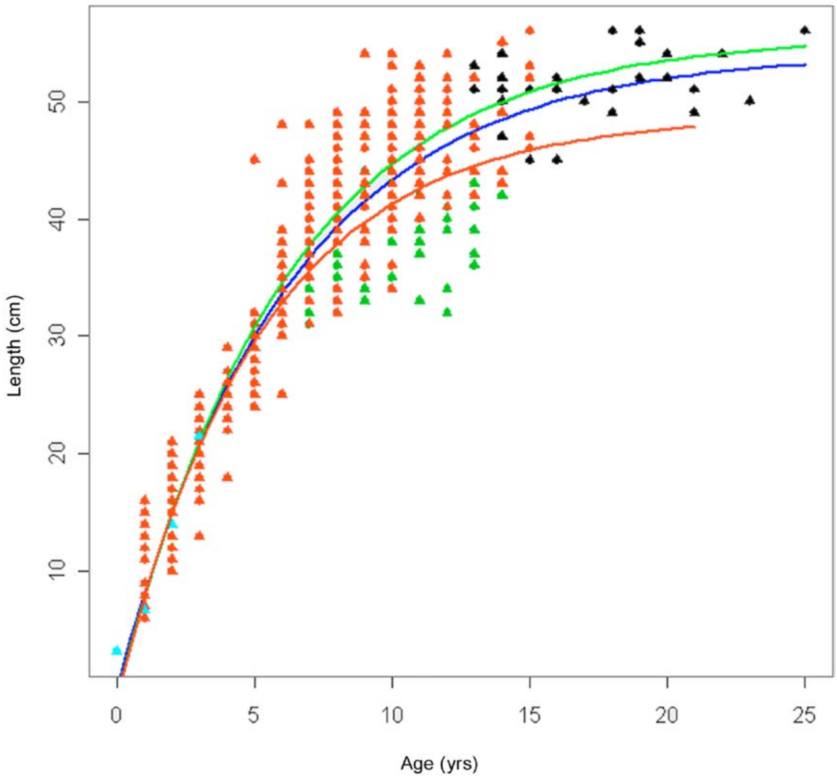

Figure 10. Yellowtail flounder length-at-age data, with fitted von Bertalanffy growth curves for females (green line) and males (red line), as well as both sexes included (blue line) using data from length-frequency (light blue symbols), sections (red symbols), tagging (green symbols) and bomb radiocarbon (black symbols). See Table 2 for curve equations.

who used edge type of scales and modal length-frequency analysis to corroborate his age readings from scales for the New England stocks. Later Lux and Nichy (1969) used tag–recapture and length-frequency modal analysis of New England yellowtail flounder stocks to validate scale reading. Neither of these methods directly validate absolute ages or annulus periodicity, and these studies were directed at young, fast-growing fish.

Length-frequency analysis was important in determining the size at which the first annulus was formed and gave information on length-at-age for the next two distinctive modes in the annual length frequencies. Yellowtail flounder spawn on the Grand Banks mainly during the summer months from May to September with a peak spawning in June (Pitt, 1970; Frank *et al.*, 1989). Young-of-year yellowtail flounder appear to settle to the bottom at a

Table 2. Summary of parameters of von Bertalanffy growth equation for yellowtail flounder from different studies. res., Research samples; com., commercial samples.

Location	Sex	Ages fitted	L_∞	K	t_o	Reference
Grand Bank res.	♂	3–11	42.07	0.41	1.39	Pitt, 1974
Grand Bank res.	♀	3–12	48.12	0.29	0.80	Pitt, 1974
Grand Bank com.	♂	4–12	46.40	0.32	0.63	Pitt, 1974
Grand Bank com.	♀	4–10	52.96	0.24	0.86	Pitt, 1974
New England com.	♂+♀	2–7	50.00	0.34	−0.26	Lux and Nichy, 1969
Scotian Shelf res.	♂+♀	4–11	52.0	0.26	1.29	Pitt, 1974
St. Pierre Bank	♂	2–8	48.38	0.15	0.50	Berthome, 1976
St. Pierre Bank	♀	2–9	56.44	0.13	0.50	Berthome, 1976
Grand Bank com.	♂+♀			0.07		Walsh and Morgan, 1999a, b
Grand Bank res.	♂	0–21	48.8	0.19	0.08	This study
Grand Bank res.	♀	0–25	55.6	0.16	0.07	This study

size between 30 and 35 mm and between mid-August and November of the year they hatch. Yevseyenko and Nevinskiy (1981) reported that, based on icthyoplankton samples, yellowtail flounder larvae in waters off New England metamorphose between 1 and 1.4 cm. Bigelow and Schroeder (1953) reported that pelagic 0-group settled to the bottom after metamorphosis. The settling size of 0-group juveniles in these southern stocks is much smaller than that estimated here ($\sim$3 cm) for the Grand Bank stock and may reflect latitudinal temperature differences in growth. Using these data on early life history stages and the modal length-frequency method, the identity of the first annulus was established. In earlier readings, this annulus had been mistaken for a settling check. Together, both methods corroborate the interpretation of the youngest age groups, at least for 0–2 years, and agree with the mean length-at-age estimated from the otoliths (Table 1). In some years, the well-defined modal lengths of strong year classes could be followed up to five peaks, but there seemed to be "mixing" of age classes within the length modes after age 3. Size selective mortality of juveniles, migration and the presence of more than one strong year class in a sample can often result in less obvious modes when tracking distinct cohorts (Meekan and Fortier, 1996; Campana, 2001).

Although growth in the laboratory is often different from that in the wild, our laboratory-reared yellowtail flounder produced only one annulus per year until age 2. This agrees with Masuda *et al.* (2000) who showed that artificially reared flathead (*Platycephalus indicus*) also only formed one annulus per year.

Marginal increment analysis supported the view that the annuli actually formed once per year in young ($\leq$7 years) yellowtail flounder. It also indicated that the annual translucent zones formed between January and April, and that the opaque zone was laid down in the summer. Campana (2001) noted that marginal increment analysis is still one of the more dubious and abused age validation methods in use today. It is only suitable for fast-growing fish, because the narrow annuli at the margin in slow-growing fish and the technical difficulties of viewing a partial increment makes this technique very difficult and subjective. For this reason, no attempt was made to use marginal increment analysis on older yellowtail.

Tag–recapture analysis was used to compare time at liberty to the number of annuli laid down after tagging. This approach was also used by Lee and Prince (1995) in their study of bluefin tuna (*Thunnus thynnus*), whereby tuna were estimated to be between 1 and 3 years old at tagging and were recaptured 15 years later. In our analysis of the otoliths from recaptured yellowtail flounder, the number of annuli laid down corresponded to the number of years after tagging up to approximately 8 years at liberty, in fish roughly 13 years of age. Thus annuli in thin sections were validated to an age of about 13 years. Beyond 8 years at liberty there was less correspondence, resulting in age underestimation. Difficulty in interpretation of the outer annuli of the thin-sectioned otoliths of older fish may be problematic and result in annuli counts that do not correspond exactly with true age as Beamish and McFarlane (2000) found in sablefish (*A. fimbria*). Apparently the same is true of yellowtail flounder.

Bomb radiocarbon assays derived from nuclear testing in the 1950s and 1960s provide one of the best validation techniques for old fish (Kalish, 1995a, b; Kalish *et al.*, 1996, 1997; Campana, 1997, 1999; Campana and Jones, 1998). The assays of yellowtail flounder otoliths hatched during the nuclear testing period showed a phase shift of $\Delta^{14}C$ towards more recent years (in Figure 9) indicating that the ages read from the thin sections underestimated the true age of fish >15 years old by about 5 years on average. The reason for this underageing may be due to the near cessation of lateral growth in older fish (>15 years). This is evident in Figure 1 which shows that the spacing between successive annuli produced after the onset of sexual maturity (M_{50} in males is age 5 and in females it is age 6; Walsh and Morgan, 1999b) tends to narrow more and more as the margin is approached. This obviously contributes to the difficulty in ageing older fish. As a result, the fact that some of the annuli in the old fish are not visible is not at all surprising. Beamish and McFarlane (2000) did not observe the expected number of annuli compared to years at liberty in some cases, leading them to believe that some annuli were being misinterpreted in oxytetracycline-tagged old sablefish. Similarly in this study, fish up to age 12 showed a strong agreement between the number of years at liberty and the number of annuli visible; however, in older fish, we also did not observe the expected number of annuli that would be commensurate with years at liberty. The bomb radiocarbon assays were completely consistent with this result, indicating that not all annuli were visible in the thin-sectioned otoliths of very old fish. Clearly, even thin-sectioned otoliths can result in age underestimation for very old and slow-growing fish. However, our results indicate that Grand Bank yellowtail flounder live longer than that reported in the literature (Pitt, 1974) and in recent assessments of this stock (Walsh *et al.*, 1999).

In many marine fish, sexual dimorphism in growth rates, size and longevity is evident, with females growing faster, attaining a larger size and having a longer lifespan than their male counterparts; this is particularly common in many flatfish species (Beverton, 1964), including yellowtail flounder (Scott, 1954; Royce *et al.*, 1959; Lux and Nichy, 1969; Pitt, 1974; this study). The difference between sexes is thought to be due to differences in the way males and females channel surplus energy into growth and reproduction. We assume that since male yellowtail flounder mature earlier, they channel energy into reproduction 2 years earlier than females and this appears to occur in Grand Bank yellowtail flounder after age 4. Males reach 50% maturity at age 4–5 years and females at age 6 years (Walsh and Morgan, 1999b). The growth rate up to age 4 is the same in both sexes. Because of this earlier slow-down in growth, males reach a smaller maximum size (50 cm) than females (56 cm) and have a shorter lifespan, i.e. 21 years vs. 25 years for females, but the overall growth rate does not appear to be different. Similar maximum sizes are seen in the commercial fishery catches (Walsh *et al.*, 2001). Lux and Nichy (1969) showed that the growth rates were identical for male and female New England yellowtail flounder up to the age of maturity, i.e. age 2 years. Interestingly, the departure in agreement in age determination between whole otoliths and thin-sectioned otoliths also occurred after sexual maturity.

In addition to sexual dimorphism in the growth patterns of male and female yellowtail flounder, the parameters of the von Bertalanffy growth curve also differ between and among geographic areas. Pitt (1974) reported that the growth parameter (K) of commercial Grand Bank yellowtail flounder was similar to that seen on the Scotian Shelf, but lower than that found by Lux and Nichy (1969) in commercial New England yellowtail flounder. The differences, they suggested, were linked to differences in temperature, with yellowtail flounder in southern stocks dwelling in warmer waters, having high growth rates and reaching maturity at a younger age than Scotian Shelf yellowtail flounder in the colder waters. Pitt (1974) also showed that the growth rates determined from his research samples were higher than the growth rates of commercial Grand Bank yellowtail flounder. Based on our age determination and validation study using survey data, Grand Bank yellowtail flounder grow much slower than Pitt's (1974) estimation of the growth rates for either research or commercial samples. The new growth parameters are similar to that reported for the St. Pierre Bank stock, off the south coast of Newfoundland and to the west of the Grand Bank, by Berthome (1976) and lower than that seen for the Scotian Shelf Stock and the New England stocks, suggesting a latitudinal cline in growth.

The new growth parameter, K (0.19 for males and 0.16 for females), for Grand Bank yellowtail flounder estimated here was higher than the growth parameter ($K = 0.07$). Walsh and Morgan (1999a) estimated from tag-recapture data, using a length-based von Bertalanffy growth equation. Walsh and Morgan (1999a) had also estimated an average growth rate of 1.7 ± 2.1 cm year^{-1} (mean $\pm$ s.e.) for yellowtail flounder based on time at liberty. In this study, the estimated average growth rate for fish in the same size range as those fish recaptured is 1.9 ± 0.5 cm year^{-1}. Comparison of growth curves from different methods can be a good way to validate ageing methods (Natanson *et al.*, 2002). However, Francis (1988) noted that the growth rates from von Bertalanffy age-length data and tagging length data are not directly comparable and that the amount of difference is unknown. Here both methods indicate that the growth rate slows down considerably after maturity. By age 15, fish are near L_∞, suggesting that growth is extremely slow at older ages. If the ageing method underestimates the true age in the oldest fish, as we suspect, then the age-based estimates of growth rate in old yellowtail flounder may still be too high and this needs to be investigated further. It is also unknown if the effects of tagging suppressed the growth in yellowtail flounder during their time at liberty. After being at liberty for nearly 2 years, tag returns from yellowtail flounder (32–36 cm at release) on Georges Bank also indicated that growth was noticeably less than predicted (H. Stone, St. Andrews Biological Station: pers. comm.). A number of other studies have questioned whether tagging or chemical marking depresses growth (example, Kelly and Barker, 1963; Saunders and Allen, 1967; MacLellan and Fargo, 1995). In addition, yellowtail flounder in the Grand Bank tag–recapture study were tagged in the early 1990s, a period of time which had uncharacteristically low temperatures (Colbourne, 1993), which could have depressed growth. The effect of tagging on growth is currently being investigated as part of a 5 year tagging program which began in 2000 (Walsh *et al.*, 2001) during a time when water temperatures on the Grand Bank had returned to warmer conditions and are closer to the long term average.

Conclusions

Age validation studies are required for accurate age determination and are essential for fisheries catch-at-age models, to assess the health of a fishery resource or to correctly interpret the dynamics of a fish population. Beamish and McFarlane (1983) examined 500 papers published from 1907 to 1980 and noted that although 66% mentioned age validation, only 3–4% actually validated all ages. Campana (2001) reviewed 372 papers published since 1983 and noted significant improvements in age validation in recent decades although some methods of dubious value were still being used. Since growth may be reduced because of sex differences and maturation it is necessary to carry out age validation over the entire range of ages. Errors in ageing may result in an accumulation of estimates in the age structure at the age in which the ageing technique fails. Such may have been the case for age determination of yellowtail flounder using whole otoliths, where older ages reported in the stock assessment were truncated at ages 9–10 during the last decade, with unexplained high total mortality estimates and growth rates estimated to be linear (see Walsh and Morgan, 1999a; Walsh *et al.*, 1999). Indeed, a similar error may have contributed to the collapse of walleye pollock fishery (*Theragra chalcogramma*) on the West Coast of Canada (Beamish and McFarlane, 1995) and decline in haddock stocks on the East Coast (Campana, 1995).

The indirect methods of validation used in this study have corroborated the accuracy of annulus interpretation in young fish. The tag–recapture results and bomb radiocarbon assays gave the best results for validating the maturing and older ages in the population. The traditional method of reading the surface of the whole otolith is appropriate for ageing Grand Bank yellowtail flounder up to 7 years, but beyond 7 years, thin-sectioned otoliths should be used to age this species. This present study is a comprehensive first look at corroborating and validating age determination in Grand Bank yellowtail flounder, using pelagic 0-group annulus measurements along with length-frequency analysis, aquarium-raised juveniles, tag–recapture analysis and bomb radiocarbon assays to provide accurate age estimates across the entire age range. In very old fish, however, it is still possible to underage due to a slow growth rate, which can produce annuli that might be misinterpreted as checks or are not visible at all. Nevertheless, yellowtail flounder on the Grand Banks live substantially longer and grow slower than previously reported in the literature (Pitt, 1974) and in recent assessments of this stock (Walsh *et al.*, 1999). These results could affect the previous understanding of commercial catch-at-age statistics, productivity, recruitment, stock assessment, maturation, reproductive potential and mortality rates. This will be the subject of further investigations.

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References

Andrews, A. H., Cailliet, G. M., and Coale, K. H. 1999. Age and growth of the Pacific grenadier (*Coryphaenoides acrolepis*) with age estimate validation using an improved radiometric ageing technique. Canadian Journal of Fisheries and Aquatic Science, 56: 1339–1350.

Beamish, R. J. 1979. New information on the longevity of Pacific ocean perch (*Sebastes alutus*). Journal of the Fisheries Research Board of Canada, 36: 1395–1400.

Beamish, R. J., and McFarlane, G. A. 1995. A discussion of the importance of ageing errors and an application to walleye pollock: the world's largest fishery. *In* Recent Developments in Fish Otolith Research, pp. 545–566. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, South Carolina, 730 pp.

Beamish, R. J., and McFarlane, G. A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112: 735–743.

Beamish, R. J., and McFarlane, G. A. 2000. Reevaluation of the interpretation of annuli from otoliths of a long-lived fish, *Anoplopoma fimbria*. Fisheries Research, 46: 105–111.

Berthome, J. P. 1976. Growth of the yellowtail flounder, *Limanda ferruginea* (STORER), fished around the St. Pierre and Miquelon Islands. International Commission for the Northwest Atlantic Fisheries, Research Document 76/VI/91.

Beverton, R. J. H. 1964. Differential catchability of male and female plaice in the North Sea and its effect on estimates of abundance. Journal du Conseil International pour Exploration de la Mer, 155: 103–112.

Bigelow, H. B., and Schroeder, W. C. 1953. Fishes of the Gulf of Maine. Fishery Bulletin, 74: 577.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Science, 47: 2219–2227.

Campana, S. E. 1995. Expert age determination of 4VW and 4X haddock otoliths by national and international laboratories. Department of Fisheries and Oceans Atlantic Fisheries Research Document, 95/120.

Campana, S. E. 1997. Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock *Melanogrammus aeglefinus*. Marine Ecology Progress Series, 150: 49–56.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263–297.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Campana, S. E., and Jones, C. M. 1998. Radiocarbon from nuclear testing applied to age validation of black drum, *Pogonias cromis*. Fishery Bulletin, 96: 185–192.

Campana, S. E., Zwanenburg, K. C. T., and Smith, J. N. 1990. ^{210}Pb/^{226}Ra determination of longevity in redfish. Canadian Journal of Fisheries and Aquatic Science, 47: 163–165.

Campana, S. E., Annand, M. C., and McMillan, J. I. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society, 124: 131–138.

Chilton, D. E., Beamish, R. J. 1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Canadian Special Publication of Fisheries and Aquatics Science, 60: 102 pp.

Colbourne, E. 1993. Oceanographic conditions during the annual fall groundfish survey in NAFO Divisions 2J3KL. NAFO Scientific Council Research Document 93/88. 31 pp.

Du, J. 1999. Combined algorithms for constrained estimation of finite mixture distributions with grouped data and conditional data. MSc thesis, Department of Mathematics and Statistics, McMaster University, Ontario, Canada.

Dwyer, K. S., Veitch, M. F., and Walsh, S. J. 2001. Age and growth of yellowtail flounder in NAFO Divisions 3LNO: an update. NAFO Scientific Council Research Document 01/52. 8 pp.

Francis, R. I. C. C. 1988. Are growth parameters estimated from tagging and age-at-length data comparable? Canadian Journal of Fisheries and Aquatic Science, 45: 936–942.

Frank, K. T., Carscadden, J. E., and Leggett, W. C. 1989. Comparative analysis of factors underlying retention of capelin and flatfish larvae on the southern Grand Banks. *In* The Early Life History of Fish. The Third ICES Symposium, Bergen, Norway, 3–5 October, 1988. Journal du Conseil International pour Exploration de la Mer, 191.

Frank, K. T., Loder, J. W., Carscadden, J. E., Leggett, W. C., and Taggart, C. T. 1992. Larval flatfish distributions and drift on the southern Grand Bank. Canadian Journal of Fisheries and Aquatic Science, 49: 467–483.

Ihaka, R., and Gentleman, R. 1996. R: a language for data analysis and graphics. Journal of Computational and Graphical Statistics, 5: 299–314.

Kalish, J. M. 1995a. Application of the bomb radiocarbon chronometer to the validation of redfish *Centroberyx affinis* age. Canadian Journal of Fisheries and Aquatic Science, 52: 1399–1405.

Kalish, J. M. 1995b. Radiocarbon and fish biology. *In* Recent Developments in Fish Otolith Research, pp. 637–654. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, South Carolina 730 pp.

Kalish, J. M., Johnston, J. M., Gunn, J. S., and Clear, N. P. 1996. Use of the bomb radiocarbon chronometer to determine age of southern bluefin tuna *Thunnus maccoyii*. Marine Ecology Progress Series, 143: 1–8.

Kalish, J. M., Johnston, J. M., Smith, D. C., Morison, A. K., and Robertson, S. G. 1997. Use of the bomb radiocarbon chronometer for age validation in the blue grenadier *Macruonus novazelandiae*. Marine Biology, 128: 557–563.

Kelly, G. F., and Barker, A. M. 1963. Effect of tagging on redfish growth rate at Eastport, Maine. International Commission for the Northwest Atlantic Fisheries, 4: 210–213.

Leaman, B. M., and Nagtegaal, D. A. 1987. Age validation and revised natural mortality rate for yellowtail rockfish. Transactions of the American Fisheries Society, 11: 171–175.

Lee, D. W., and Prince, E. D. 1995. Analysis of otoliths and vertebrae from nine tag-recaptured Atlantic bluefin tuna (*Thunnus thynnus*). *In* Recent Developments in Fish Otolith Research, pp. 361–374. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, South Carolina. 730 pp.

Lux, F. E., and Nichy, F. E. 1969. Growth of yellowtail flounder, *Limanda ferruginea*, (Storer) on three New England fishing grounds. International Commission for the Northwest Atlantic Fisheries, 6: 5–25.

MacLellan, S. E., and Fargo, J. 1995. Validation of age and growth for English sole (*Parophrys vetulus*) in Hecate Strait, British Columbia. *In* Recent Developments in Fish Otolith Research, pp. 341–360. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, South Carolina. 730 pp.

McFarlane, G. A., and Beamish, R. J. 1995. Validation of the otolith cross-section method of age determination for sablefish (*Anoplopoma fimbria*) using oxytetracycline. *In* Recent Developments in Fish Otolith Research, pp. 319–330. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, South Carolina. 730 pp.

Masuda, Y., Ozawa, T., and Onoue, O., and Hamada, T. 2000. Age and growth of the flathead, *Platycephalus indicus*, from the coastal waters of west waters of west Kyushu, Japan. Fisheries Research, 46: 113–121.

Meekan, M. G., Fortier, L. 1996. Selection for fast growth during larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. Marine Ecology Progress Series, 137: 25–37.

Morgan, M. J., and Walsh, S. J. 1999. An update of results of tagging experiments with juvenile yellowtail flounder in NAFO Divisions 3NO. NAFO Scientific Council Research Document 99/23. 8 pp.

Murphy, M. D., and Taylor, R. G. 1991. Direct validation of ages determined for adult red drums from otolith sections. Transactions of the American Fisheries Society, 120: 267–279.

Natanson, L. J., Mello, J. J., and Campana, S. E. 2002. Validated age and growth of the porbeagle shark, *Lamna nasus*, in the western North Atlantic Ocean. Fisheries Bulletin, 100: 266–278.

Pearson, D. E., Hightower, J. E., and Chan, J. T. H. 1991. Age, growth and potential yield for shortbelly rockfish, *Sebastes jordani*. Fisheries Bulletin, 89: 403–409.

Petersen, C. G. J. 1892. Fiskensbiologiske forhold I Holboek Fjord, 1890-91. Beret. Danske Biologisk Station 1890-91, 1: 121–183.

Pitt, T. K. 1970. Distribution, abundance, and spawning of yellowtail flounder, *Limanda ferruginea*, in the Newfoundland area of the Northwest Atlantic. Journal of the Fisheries Research Board of Canada, 27: 2261–2271.

Pitt, T. K. 1974. Age composition and growth of yellowtail flounder (*Limanda ferruginea*) from the Grand Banks. Journal of the Fisheries Research Board of Canada, 31: 1800–1802.

Report of the Canada–United States yellowtail flounder age reading workshop, November 28–30, 2000, St. John's Newfoundland. Walsh, S. J., and Burnett, J., (eds) 2001. NAFO Scientific Council Research Document 01/54. 57 pp.

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

Saunders, R. L., Allen, K. R. 1967. Effects of tagging and fin-clipping on the survival and growth of Atlantic salmon between smolt and adult stages. Journal of the Fisheries Research Board of Canada, 24: 2595–2611.

Scott, D. M. 1947. The biology of the yellowtail flounder (*Limanda ferruginea*-Storer). MSc thesis, McGill University, Montreal, Canada.

Scott, D. M. 1954. A comparative study of the yellowtail flounder from three Atlantic fishing areas. Journal of the Fisheries Research Board of Canada, 11: 171–197.

Scott, W. B., and Scott, M. G. 1988. Atlantic fishes of Canada Canadian Bulletin of Fisheries and Aquatic Science, 219. University of Toronto Press, Toronto, Canada, 731 pp.

Stuiver, M., and Polach, H. A. 1977. Discussion: reporting of 14C data. Radiocarbon, 19: 355–363.

Walsh, S. J. 1992. Factors influencing distribution of juvenile yellowtail flounder on the Grand Bank of Newfoundland. Netherlands Journal of Sea Research, 29: 193–203.

Walsh, S. J., and Morgan, M. J. 1999a. New information on age and growth of yellowtail flounder in Div. 3LNO as determined from tag returns. NAFO Scientific Council Research Document 99/54. 18 pp.

Walsh, S. J., and Morgan, M. J. 1999b. Variation in maturation of yellowtail flounder (*Pleuronectes ferruginea*) on the Grand Bank. Journal of Northwest Atlantic Fishery Science, 25: 47–59.

Walsh, S. J., Brodie, W. B., Morgan, M. J., and Power, D. 1999. The 1999 assessment of Grand Bank yellowtail flounder stock in NAFO Divisions 3LNO. NAFO Scientific Council Research Document 99/68. 37 pp.

Walsh, S. J., Morgan, M. J., Power, D., and Darby, C. 2001. The complete 2001 assessment of grand Bank yellowtail flounder stock in NAFO Divisions 3LNO. NAFO Scientific Council Research Document 01/76. 72.

Whalen, K., Veitch, M., and Walsh, S. J. 2000. Age determination in yellowtail flounder on the Grand Bank: a new approach. NAFO Scientific Council Research Document 00/32. 16 pp.

Yevseyenko, S. A., and Nevinskiy, M. M. 1981. On the development of the eggs and larvae of the yellowtailed dab *Limanda ferruginea* (Storer) and their passive transport in the Northwestern Atlantic. Journal of Ichthyology, 4: 65–74.