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Analysis of Age Validation Studies in Yellowtail Flounder

by

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Abstract

Traditionally, stocks of yellowtail flounder on the southern Grand Bank are aged by reading the surface of the whole otolith. However, returns from tagging studies in the early-1990s showed that there was underestimation in the ageing. A comparison of ageing methods revealed that age estimates from thin sections gave the oldest ages after the age of 7, compared to the traditional whole otolith method, the baked thin section method and ages derived from scales. However, the accuracy of ages derived from these methods had not been quantitatively validated. In this paper, we examine several methods of indirect age validation to validate methods for the youngest and middle ages of yellowtail flounder, and two direct validation methods to validate the ageing of older fish. The latter includes tag-return analysis and bomb radiocarbon assays. It is concluded that even thin-sections may underestimate the oldest fish in the population, but that this method is the most accurate. It is recommended that a yellowtail flounder reference collection be set up based on the known aged fish from bomb radiocarbon assays.

Introduction

Age validation of a particular age reading method is an absolute requirement in fisheries science. Validation is a process that provides accurate age confirmation of the ageing method. Without age validation studies, it cannot be stated for certain whether a method gives the true age of a fish, nor is there certainty whether there is one annulus formed per year. All ages for every species should be validated in order for a technique to be routinely used for ageing fish and thereby obtaining information on growth rates, mortality and the ecological importance of longevity and life history. Errors in any of these will lead to mismanagement of the fishery. In 1983, Beamish and McFarlane pointed out how validation studies are often taken for granted in fisheries biology and the consequences of doing so. These consequences include: overestimating mortality, masking the importance of strong year classes, failing to detect differences in growth rates, and misunderstanding of life history patterns, all of which could have detrimental effects on estimating the health of any resource.

Validation studies are carried out using a number of different methods, some of which may be more scientifically valuable than others and these may be either direct or indirect. Direct methods involve the use of known age or partially known age fish, whereas indirect methods estimate age by measuring the frequency of occurrence of various parameters. Indirect methods include determining the first annulus, length-frequency analysis, captive rearing of fish from hatch and marginal increment analysis. Direct methods include releasing known-age marked fish into the wild, mark-recapture of chemically tagged wild fish and bomb radiocarbon assays.

Bomb radiocarbon assays are thought to be one of the best age validation approaches for long-lived fish (Kalish, 1995a; Campana, 1997, 1999, 2001(in press)). A sharp increase in the amount of radiocarbon in the 1950s resulted in a time reference, which is reflected in the uptake of radiocarbon in the world's oceans. By comparing this time line with the amount of radiocarbon in otoliths of fish hatched during that time period, accurate ages can be validated in this manner. This method has been used to validate a number of long-lived fish, such as haddock (*Melanogrammus aeglefinus*) (Campana, 1997), black drum (*Pogonias cromis*) (Campana and Jones, 1998), redfish (*Centroberyx affinis*) (Kalish, 1995b), bluefin tuna (*Thunnus maccoyii*) (Kalish *et al*., 1996) and blue grenadier (*Macruronus novaezelandiae*) (Kalish *et al*., 1997).

Yellowtail flounder on the Grand Bank (NAFO Div. 3LNO) were assumed to be a short-lived, fast-growing species based upon the readings of whole otoliths (Pitt, 1974). Whalen *et al*. (2000) used thin section technique of otoliths and showed that yellowtail flounder are older and grow more slowly than determined from whole otolith age interpretations. However, neither of these ageing methods has been validated. In this paper, we examine the results of both indirect and direct validation studies carried out to measure the accuracy of ageing yellowtail.

Materials and Methods

A. Indirect Age Validation

Length-Frequency Analysis

The Petersen method plots numbers of fish at length to produce distinct modes along the x-axis, which represent the early ages and therefore the young year classes (Hilborn and Walters, 1992). Modal progression of strong year classes also aid in determining age groups.

Data Sources

Length frequencies of 0 group yellowtail flounder were determined from data collected in annual ichthyoplankton surveys (pelagic 0 group surveys) (1994-99). Historical data (1986-88) from surveys investigating environmental conditions on the distribution of several demersal and pelagic species' larvae (Frank *et al*., 1992) during September were examined (Fig. 1).

Length-frequency on demersal juveniles was obtained from annual DFO juvenile groundfish surveys that ran from 1985-1994 and are separated by sex. These data are most representative of the total age composition of yellowtail flounder in the demersal stage (Fig. 2 and 3). Average lengths were found by taking the upper length of a mode, subtracting the lower length of the mode and dividing by 2.

Determining the First Annulus

The diameter of fish <8 cm captured in the spring surveys, presumably when the annulus was laid down, were measured by using the Optimas v. 6.2 image analysis system. Diameter was taken across the widest part of the otolith, along the axis it was read. Diameter was recorded in millimetres.

Captive Rearing of Yellowtail Flounder From Hatch

Yellowtail flounder were reared from eggs hatched at the Ocean Sciences Centre in Logy Bay, Newfoundland. They were kept in a flow-through facility, at heated temperatures and fed a commercial diet. Both 1+ (n = 12) and 2+ (n = 12) fish were killed and the otoliths removed for examination. An age reader read the ages from both whole otoliths and then later, from thin sections, and these were compared to the actual ages of the fish.

Marginal Increment Analysis

Yellowtail flounder were captured during seasonal groundfish surveys of the Grand Bank throughout the years 1985-86. The left otolith was removed and sectioned using a low-speed Isomet saw with double blades, removing a 0.5 mm transverse section. These otoliths are immersed in alcohol and read under reflected light and then again by the same ager, so that an acceptable level of precision was reached. The reader had no knowledge of length, sex or time of capture.

The marginal increment is defined as the width of the inner edge of the outermost opaque zone and the periphery, and is expressed as a proportion of the width between the inner edges of the two outermost opaque zones. This was done using Optimas v. 6.2 image analysis system and taking two measurements at 40 X magnification. Annuli were counted along the dorsal edge of the otolith and followed along to the sulcus acusticus where possible. Ages were pooled into groups (ages 3-7 annuli and 8+ annuli) because of low sample sizes. In addition, because fish were not collected in every month, months were pooled into January/February, April/May, July/August and October/November to represent seasons.

B. Direct Age Validation

Tag-Recapture Analysis

Yellowtail flounder were tagged and released on the southern Grand Bank (Morgan and Walsh, 1996) in the early-1990s as juveniles (<30 cm). Because these fish were juveniles, age-at-release could be estimated using back-calculation equations, such as the Fraser-Lee method:

$$L_a = d + (L_c - d)\ O_c - 1\ O_a$$

where L is fish length at some previous age (a), measured size of the otolith (O) at age a and d is the intercept of the fish length-otolith measurement regression (in Campana, 1990).

Thin sections of the otolith from the recaptured fish were prepared as before, and read in the same manner (see Whalen *et al*., 2000, for details). Annulus age was based on repeated counts by Ager 2. The radius of each section was then measured across from the primordium, along the dorsal tip to the outer edge of the otolith. The relationship between fish length and otolith radius was constructed using tagging samples and juveniles in order to find d, the y-intercept. Once constructed, the equation was solved for O_a and the number of annuli after this radius could be compared with time at liberty and an age bias plot was constructed (Campana, 1995) to determine whether there were any differences. The age-at-release estimate could be added to the time at liberty for each tag-recaptured fish in order to determine the actual age of the fish.

Bomb Radiocarbon Assay

Annulus age was estimated from one or both sagittal otoliths of each pair. Thin sections (~1 mm thick) of each otolith were prepared by sectioning transversely through the core. Annulus age was based on repeated counts by one of the co-authors (KD, Ager 2) from digitally enhanced images photographed under reflected light. The radius of the presumed first annulus was confirmed through measurements of the dimensions of intact sagittae collected from young of the year yellowtail, which indicated that the first annulus of a transverse section should be approximately 0.75 mm wide (see previous section). After ageing, the remaining halves 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 two 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 core sample weights were insufficient for individual assay, cores were isolated from each otolith of the pair and pooled. Most samples were also pooled with one other fish of similar age and hatch date, so as to bring total sample weight to at least 7 mg. All core material was then decontaminated (Campana 1999), wrapped in aluminium foil and submitted for ^{14}C assay by AMS. 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 per mil ($^0/_{00}$) 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 core assays can be compared. Uncertainty around the reference line is no more than two years between 1957 and 1965.

Results and Discussion

A. Indirect age validation

Length-Frequency Analysis (from Whalen *et al*., 2000)

Ichthyological surveys revealed yellowtail flounder larvae in the water in late August early September that must have been newly hatched larvae from that spring/summer. These larvae were between 4-34 mm in length at this time (see Fig. 1).

DFO groundfish surveys do not pick up any 0+ group yellowtail flounder, except in the male fish of November, 1987 (Fig. 2). These fish have a modal length of 3 cm, which are newly settled juveniles, and the next mode, approximately 6-9 cm length, should indicate the 1group that year. This strong year class can be followed for the next couple of years. The next group, the 2-year-old fish, are about 12 cm in length (Fig. 2 and 3). However, beyond this group, it is difficult to distinguish modes.

Thus this method validates the youngest age groups of yellowtail flounder and it is thought that this will add to the information on interpretation of the first annulus. It is noted however, that this method is only useful for the youngest (0-2 years) and fastest growing fish.

Determination of the First Annulus

The first annulus had an average diameter of approximately 0.65 mm. It was difficult to obtain 1-year old fish at this time of year, so known-age (from length frequency analysis) 2-year-old fish were examined. The first annulus is consistently about 0.65-0.7 mm and between 0.3 to 0.4 mm thick. However, when the same measurements were taken from otoliths in older fish, the measurements for first annulus were larger (0.75 mm) but comparable, indicating that this annulus is indeed the first annulus. Although smaller in younger fish, the first annulus may have been compromised in size due to colder water temperatures.

Previous problems that had occurred with interpreting the first annulus (Whalen *et al*., 2000; Dwyer *et al*., 2001) should be resolved in future ageing by this work.

Captive Rearing of Yellowtail Flounder

When compared, there were no differences in estimated age and known age of the otoliths from captive fish for the first and second years of life (see Fig. 4). In captive fish, there seems to be a strong settling check, which might be taken under consideration when interpreting the first annulus in wild yellowtail.

Although the conditions in which captive yellowtail are reared do not resemble the conditions in the wild in any way, this does show validate one annulus is laid down per year under optimal growing conditions.

Marginal Increment Analysis

From Fig. 5, it is noted that there is a pattern in the cycle of marginal increment in the 3-7 year-old fish, but it is not meaningful. At no point is there fully 1.0 marginal increment, and this pattern is even less obvious in 8+ fish. Therefore the marginal increment analysis performed on these fish is inconclusive, although it should be stated that there was virtually no pattern in marginal increments from whole otoliths.

Tag-Recapture Analysis

The age bias plot in Fig. 6 shows that there is no bias between annuli estimated after tagging and time at liberty. There does seem to be some underestimation at older ages, which might be explained by the primary reader not including the marginal edge when ageing. In addition the CV is high, at 11.4%. However, a paired *t*-test with p-value 0.6094 also shows there is no significant difference between the two, indicating that the annuli estimated from thin sections for these tagging returns represent the true ages of the fish (see Table 1).

It is noted that the length at age of tag recaptures was much lower than expected, indicating very slow growth, even with correct ages. This may be due to the fact that the time period in which these fish were tagged corresponds to historically low coldwater temperatures of the early-1990s (Colbourne, 1993).

Bomb Radiocarbon Assays

The date of formation of the yellowtail otolith cores was estimated in two ways: through age determination based on annulus counts, and through comparison of otolith core $\Delta^{14}C$ values with the values known to be present in the marine environment at the time. Previous studies have clearly demonstrated that the rapid increase in marine $\Delta^{14}C$ due to atmospheric testing of nuclear weapons was widespread and synchronous throughout the Northwest Atlantic, and was reflected in otoliths, corals and bivalves growing at the time (Campana 1997; Campana and Jones 1998). Therefore 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 yellowtail 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 4.7-5.3. There was no significant relationship between $\delta^{13}C$ and either presumed age or hatch date; mean $\delta^{13}C$ was –2.51 (SE = 0.13).

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 (Fig. 7). However, comparison with the accurately dated reference $\Delta^{14}C$ curve indicates that most of the annulus-based age determinations cannot be correct. The yellowtail assay results are offset by an average of about 5 years compared to the reference curve, indicating that the annulus counts were underaged (on average) by about 5 years. Since $\Delta^{14}C$ values set a minimum age for a sample, and since coring error can only reduce the apparent age of the sample and not increase it, there is no explanation for the discrepancy other than annulus count underestimation.

All of the otoliths aged for radiocarbon assay were from old fish: the range of annulus-based ages was 13-25 years, and the radiocarbon results indicate that they must be even older. Age reader 2 tended to over-age slightly compared to the primary age reader for most ages, but not sufficient enough to remove the underageing bias (Fig. 7). The detailed examination of each otolith indicates that lateral growth (in the transverse section) ceases completely in older fish, typically between 15-20 years. All subsequent growth occurs on one or both sides of the sulcus, and is very small. As a result, the fact that some of the annuli in the really old fish are not visible is not at all surprising. This is evident in high-magnification images (Fig. 8), which show that the annuli produced after the onset of sexual maturity tend to be quite narrow and this contributes to the difficulty in ageing older fish. This is an interesting and important point, and is consistent with McFarlane and Beamish's (2000) observations of the same phenomenon in old OTC-tagged sablefish. Clearly, even thin-sectioned otoliths can result in age underestimation for very old and slow-growing fish.

Given the fact that the underageing was first apparent at age 15, we would suggest that this is a logical endpoint for the catch at age matrix, i.e. 15+ as the plus group. This can be verified by plotting length *vs* age for all fish used in this study (age bias, tagging and bomb). We suspect that the curve will flatten out almost to an asymptote in the 17+ fish, thus explaining why otolith growth after this age is so limited.

Conclusions

The correct interpretation of annuli is often taken for granted in age reading and errors can lead to over- or underestimations of population parameters. Confirmation of the accuracy of age reading is a difficult and time-consuming process. Some of the indirect methods of validation used in this study have confirmed the accuracy of annulus interpretation in young fish. Both tag-recapture analysis and bomb radiocarbon assays gave the best results for validating the oldest ages in the population. The assay work has shown that ages read from the thin sections underestimate the oldest fish by about 4-5 years, and similar difficulty in interpretation of the thin outer annuli was seen by Beamish and McFarlane (2000). Although difficulty exists in ageing the oldest fish, such known-age fish from this study under the ages of 15+ may be used for training and reference collections by inserting them randomly in image archives of otolith sections (Walsh and Burnett, 2001).

The information from the age validation studies in this paper reveal a very different life history for Grand Bank yellowtail flounder, and different age and growth patterns than that described by Pitt (1974) for this stock. This will affect growth rates, reproductive potential, mortality rates and may influence age at maturity, as well as other, more subtle life history parameters.

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Table 1. Mean length-at-age (cm) aged by the "true age" (estimated age at tagging plus time at liberty) of tag-recapture fish.

"True" Age	Length-at-age (cm)
7	34.1
8	32.8
10	38.0
11	38.2
12	39.3
13	38.3
14	35.5
15	41.0

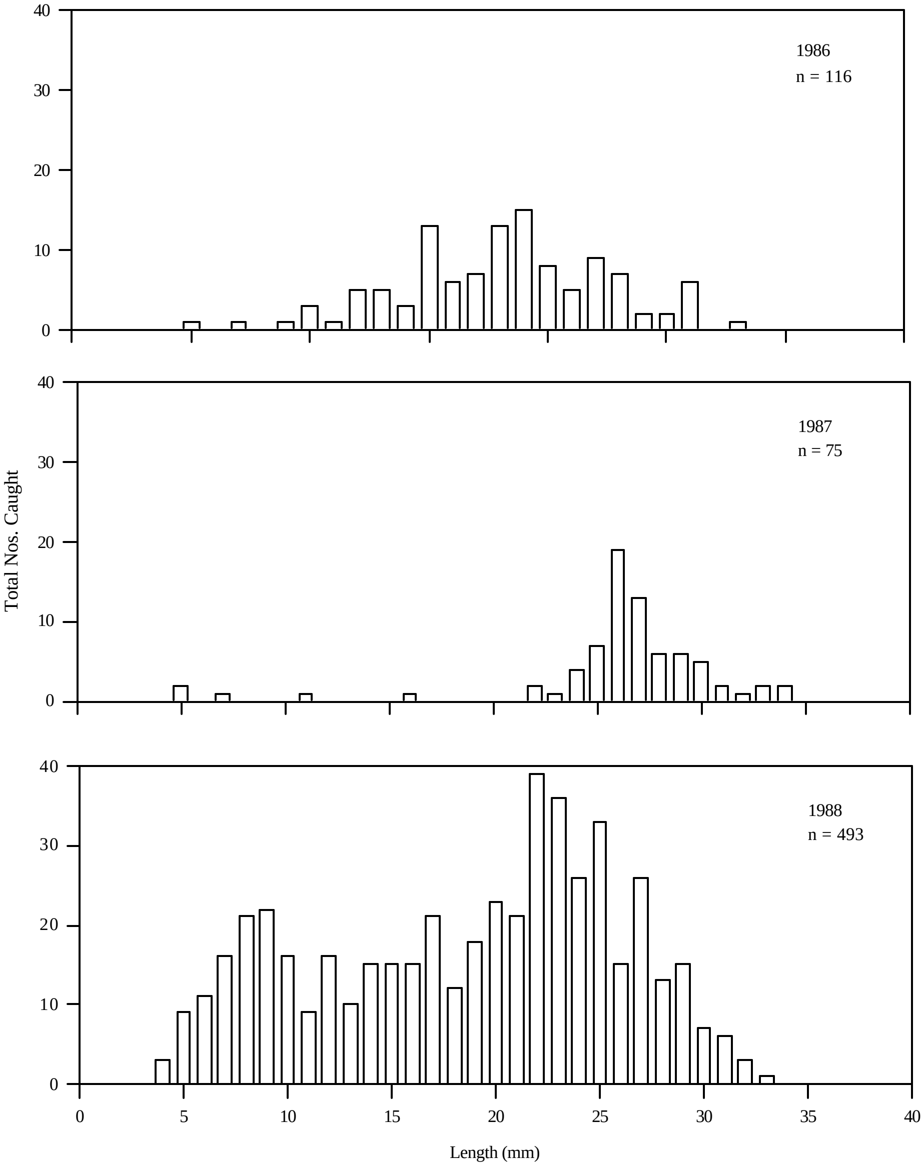

Fig. 1. Length compositions (mm) of pelagic yellowtail flounder larvae from surveys conducted on the southern Grand Banks during September for 1986-1988 (*n* = total number caught). From Frank *et al*. (1992), used with permission of J. Carscadden, DFO, NF.

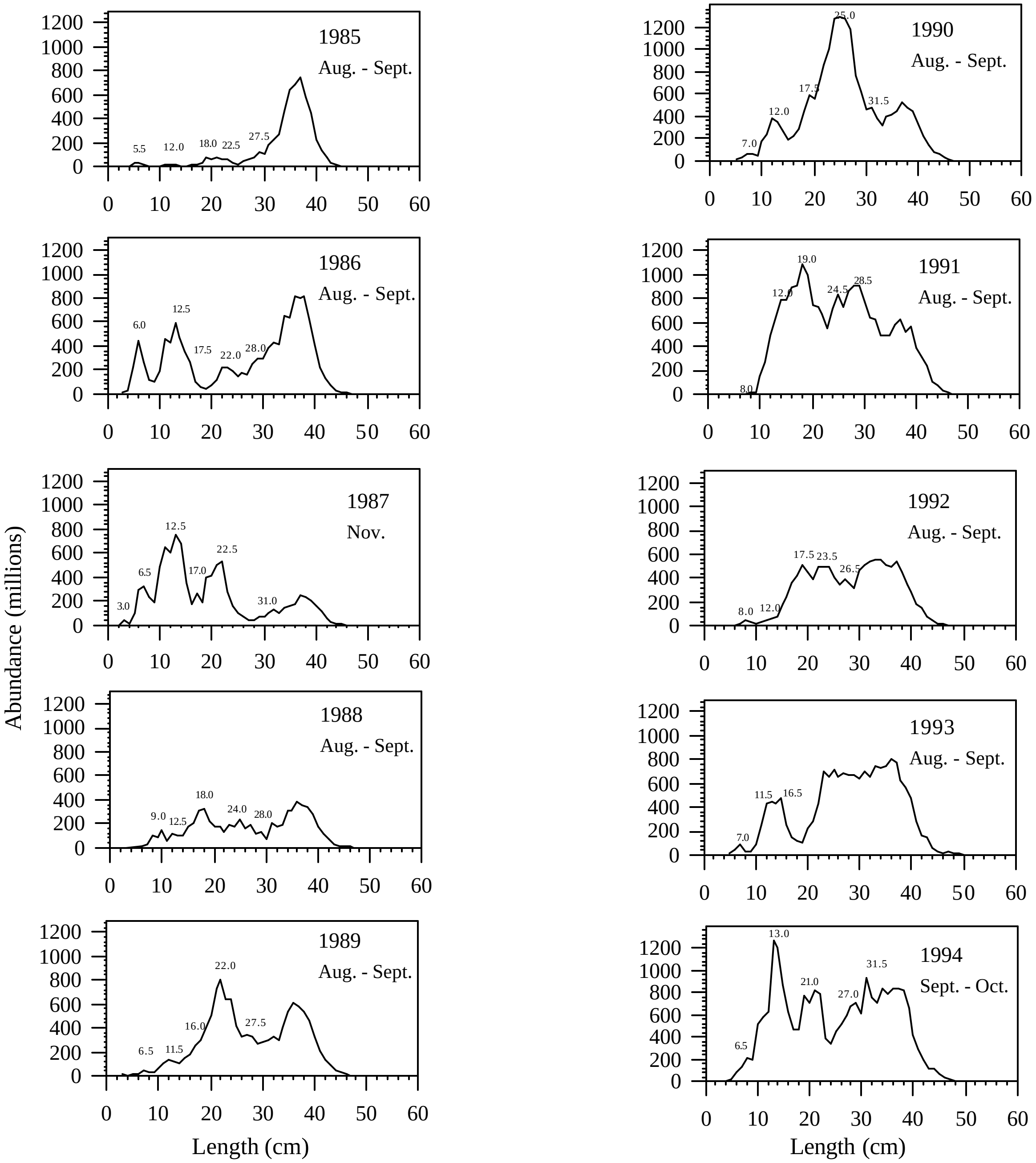

Fig. 2. Length compositions (cm) of male yellowtail flounder from annual DFO juvenile groundfish surveys from 1985-1994. Time of year is indicated. Numbers are average length of modes (cm).

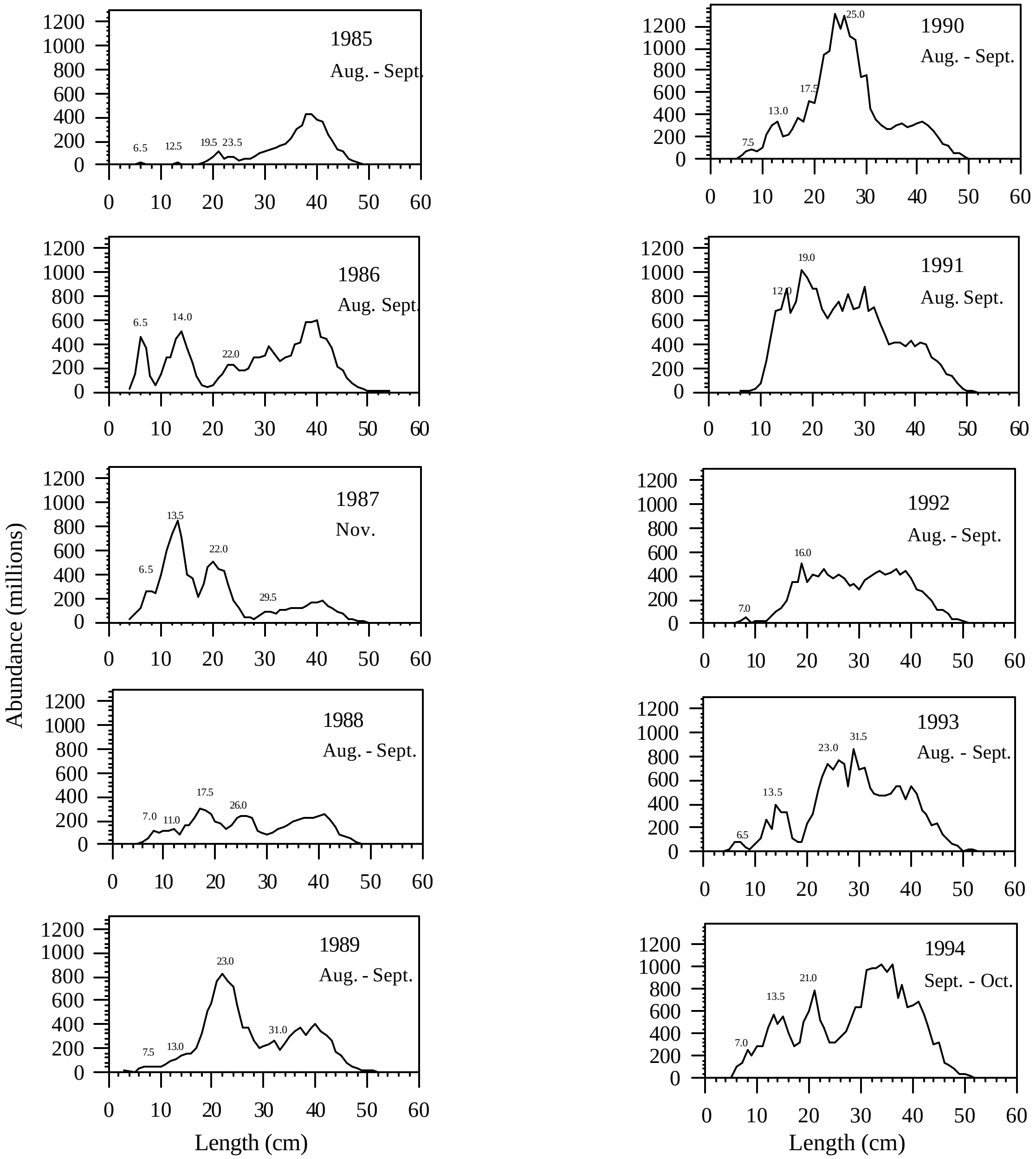

Fig. 3. Length compositions (cm) of female yellowtail flounder from annual juvenile groundfish surveys from 1985-1994. Time of year is indicated. Numbers are average lengths of modes.

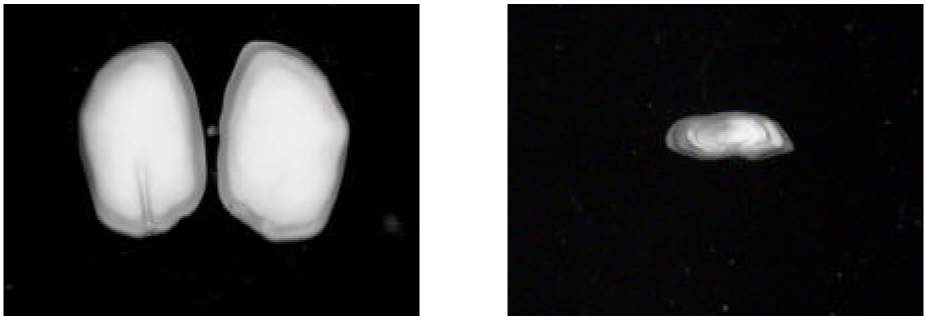

Fig. 4A. Otoliths removed from 1+ yellowtail flounder reared in captivity. Fish were killed in April.

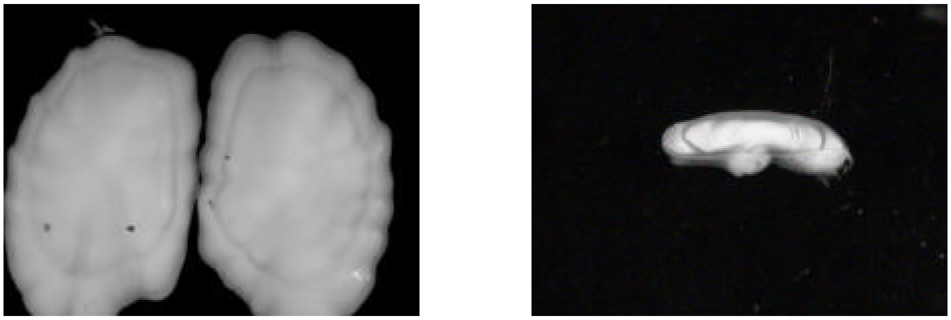

Fig. 4B. Otoliths removed from 2+ yellowtail flounder reared in captivity. Fish were killed in April.

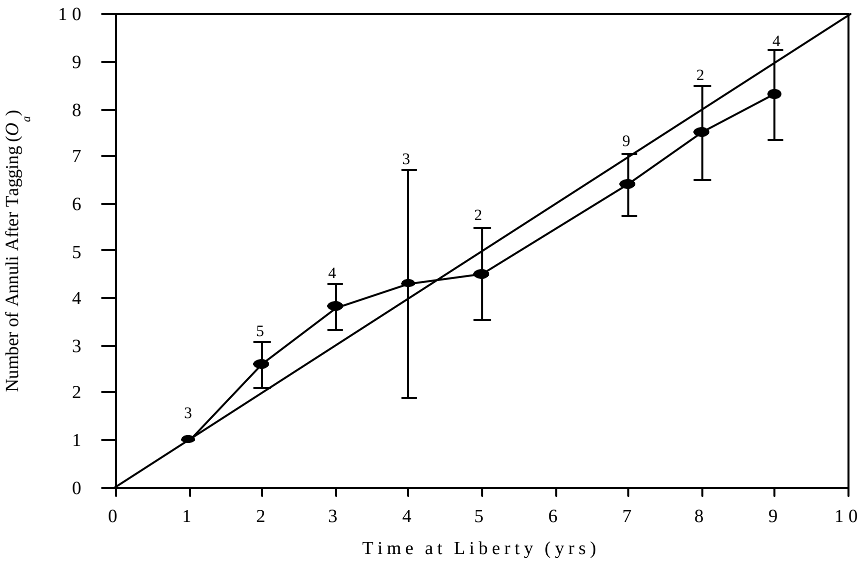

Fig.5. Monthly changes in mean marginal increment for sectioned otoliths of yellowtail flounder. Numbers indicate sample size.

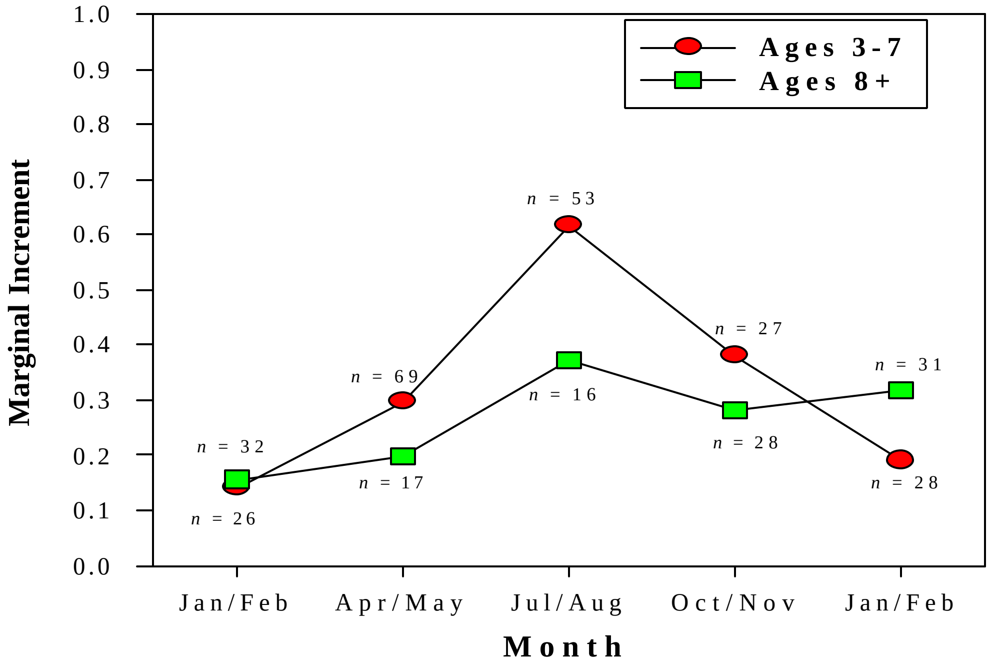

Fig. 6. Age bias plot showing the difference between time at liberty for tag-recaptured yellowtail flounder and the number of annuli after tagging, estimated from a fish-length and otolith radius regression, using the Fraser-Lee equation. Each error bar represents the 95% confidence interval about the mean time at liberty assigned for one otolith for all fish assigned a given number of annuli after tagging for the second otolith. The 1:1 equivalence (solid line) is also indicated. Numbers plotted with symbols are the sample size for each.

Fig. 7. $\Delta^{14}C$ concentrations in the otoliths of old yellowtail flounder in relation to published $\Delta^{14}C$ chronologies for redfish (*Sebastes* spp) and haddock (*Melanogrammus aeglefinus*) (Campana 1997).

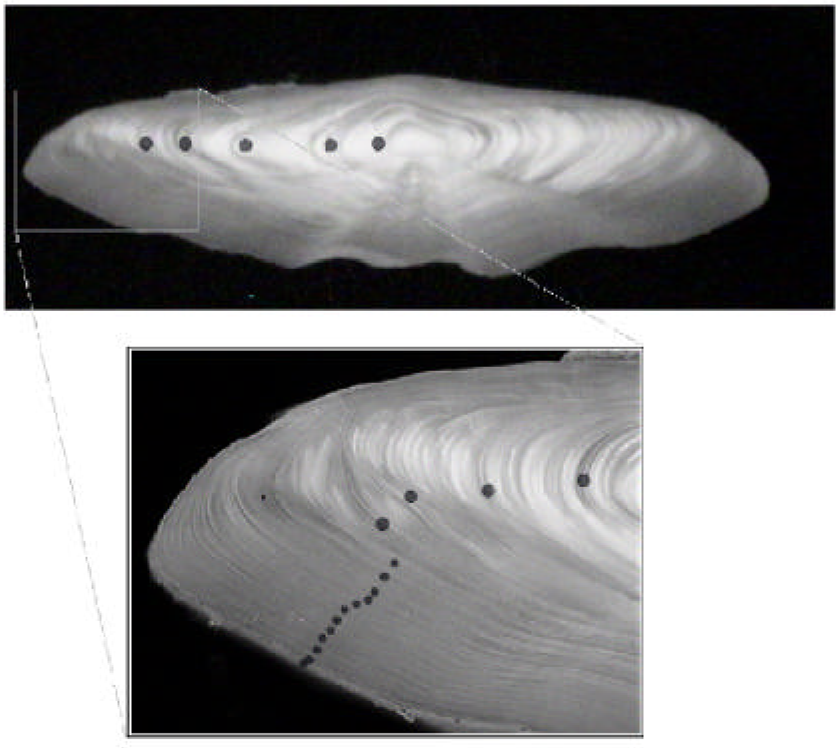

Fig. 8. Transverse section of a yellowtail otolith, revealing the annuli. Annuli formed before sexual maturation (top insert) look very different than those formed after sexual maturation (bottom insert).