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Age Determination in Yellowtail Flounder on the Grand Bank: A New Approach

by

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Abstract

The standard age reading method was compared with a new method for ageing yellowtail flounder at the Northwest Atlantic Fisheries Centre. The standard method of reading whole otoliths was found to be inadequate after the age of 7 years old. Length-frequency analysis gave estimates of length at age for the first five years for yellowtail flounder. Age estimates of new method of age reading thin sections of otoliths gave a maximum age of 16 years to female yellowtail and 13 years to male yellowtail. Age validation is advised, and radiocarbon dating from nuclear fallout is recommended.

Introduction

Age determination in fisheries is vital to the successful management of a fish stock. It enables scientists to know the age composition of a population of fish, and thereby make decisions based on mortality, recruitment and assign catch quotas accordingly. In temperate climates, most ageing is done through the use of hard parts such as otoliths or scales. Other structures which may be used are vertebrae, fin rays and spines, but these are less common. These hard parts contain a record of growth for the fish, and by counting periodic rings, or annuli, readers can determine the age of the fish. It is important to know whether these rings actually represent a definite period of growth (annual or daily), and therefore, one must carry out age validation. However, this is a time-consuming and expensive process, and one that may be extremely difficult for older, long-lived fish. Comparison of various methods is not an accurate validation technique, but it is a starting point to determine if the methods are precise, and whether further work must be carried out to validate.

Yellowtail flounder on the Grand Bank (NAFO Division 3LNO) were assumed to be a short-lived, fast-growing species (Pitt, 1974), but recent tagging studies indicate that they may grow more slowly and live longer than currently being determined from whole otolith age interpretations (Walsh and Morgan, 1999). Such findings change our perceptions of this population and misleading results could occur if age determination should prove to be incorrect. At the NAFO Scientific Council Meeting, June 1999, Council recommended that "priority be given to restore the Council's ability to do age-structure analysis on this stock" (NAFO 1999). This paper represents the first step in the process to meet that goal.

Materials and Methods

In an attempt to understand if there was a problem in age reading of yellowtail flounder otoliths, the first step in the age determination process was to compare the readings done by the 'traditional' method of reading whole otoliths (surface reading) with a 'new method' of sectioning otoliths (internal reading).

Part I. Differences in age of yellowtail flounder using whole and sectioned otoliths

Part A

Both sagittal otoliths were collected from 204 yellowtail flounder in NAFO Divisions 3LNO during annual surveys in 1999. These were removed and stored dry. At the Northwest Atlantic Fisheries Centre (NAFC) in St. John's, there is only one 'primary' ager. For this study a 'new' age reader was trained by the primary reader to permit comparisons of age readings. All otoliths were aged twice by the two readers, without any additional information on length, sex or time of capture. Readings were done in a random order. After the otoliths were read, the two otoliths were sectioned, so that choice of otolith was not confounded with the method used. Otoliths were sectioned by embedding an otolith in wax and using an Isomet low-speed macrotome saw to remove a section approximately 0.5-0.9 mm thick. This was done using a transverse cut through the nucleus, along the dorsolateral plane of the otolith. Because of the brittle nature of smaller otoliths, otoliths from fish less than 30 cm were cut through the nucleus and each half of the otolith was examined.

Whole otoliths were placed in a methyl alcohol chamber against a dark background and examined microscopically under reflected light (Nikon dissecting microscope) at about 8-20X magnification. Rings of otoliths consist of two growth zones, the opaque (representing summer growth) zone and the hyaline, or transparent (representing winter growth) zone. The number of hyaline zones (annuli) was recorded. Sectioned otoliths were also immersed in alcohol as well, against a dark background at magnifications of 25-40X with reflected light.

Readers, structures and methods were compared using age bias plots to assess the extent of any differences between these variables, and to test for accuracy (Campana *et al*., 1995). For each paired comparison, a coefficient of variation (CV) was used to measure precision and a paired t-test was used to compare differences statistically. A general linear model was used to compare all variables simultaneously.

Part B

Traditionally, at NAFC Institute, otoliths that are difficult to read are ground to reveal annuli. These otoliths are 'cloudy' and the annuli hard to distinguish. To determine the effect this has on ageing, and therefore to ensure method comparisons were not biased, a sub-study was done to compare ages from whole unground and whole ground otoliths. 100 otoliths were chosen from divisions 3LNO, across all length groups and both sexes.

All left and right otoliths were aged by two readers. The left otoliths were then ground on the sulcus (grooved side of the otolith) face and aged again. The right otoliths were ground on the flat surface and then aged again. The grinder consisted of a two-sided (coarse side, fine side) carborundrum stone mounted on a flat base. It was powered by a sewing-machine motor and operated by use of a foot pedal. The otolith surface to be ground was laid on the stone surface and held there for approximately thirty seconds while the stone rotated, or until the reader judged the rings to be more clear. Readings were compared using age bias plots and t-tests, as above.

The second step was to examine other sources of information to validate ageing derived from otoliths.

Part II. Length Frequency Analysis

Length frequency

When the numbers of fish at various lengths are plotted, usually a series of distinct peaks will result. It is assumed that these peaks, or modes, represent distinct age groups within a population. This graphical representation method, also known as the Petersen method, can be used to validate young year classes, which tend to grow quickly, and thus show very distinct peaks (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.

Length-frequency of demersal juveniles were 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.

Length-frequencies were plotted for data collected during annual fall surveys from 1990 to 1998, and represent mainly the older age classes, although some younger modes are represented. These data are used to corroborate the other data from juvenile groundfish surveys used in the previous analysis. These are also separated by sex. Average lengths were found by taking the upper length of a mode, subtracting the lower length of the mode and diving by 2.

Results

Part I *Comparison of Methods*

Whole Otolith Readings of Left and Right Otoliths

The level of precision in ageing between the two age readers of whole otoliths gave a fairly high average coefficient of variation (CV) of about 9.7% for the left otolith and 11.0% for the right otolith. The high CVs were thought to be due to the differences in the experience levels of the two readers. However, there was no evidence of major systematic bias between readers for either the left or the right otoliths, and the age readings were not statistically different between readers ($p = 0.353$). Any bias that existed was between $\pm$ 0.75 years.

It became obvious, however, that there was bias in the reading of the orientation of otoliths. In all cases, both readers tended to "underage" the right otolith at older ages (Figure 1 a and b). This may be due in part to the asymmetry of the right otolith. Because it is thicker and more irregular than the left otolith, annuli can often be overlooked, especially at the older levels. This difference in the ages estimated for the left and right otolith was statistically significant ($p = 0.021$).

Ground Otolith Readings versus Unground Otolith Readings

Again, the CV between the two readers for the left whole otolith was fairly high, at 9.2% and between two readers for the right otolith, the CV was 8.8%. There was no bias detected by age bias plots between readers, left or right otoliths or for ground and unground otoliths (Figure 2). A general linear model (nested ANOVA) revealed that there were no statistical differences between estimated ages for reader ($p = 0.052$), orientation (left/right otoliths) ($p = 0.594$) or method (ground/unground) ($p = 0.900$).

Sectioned Otolith Readings

The CV between readers for the left (14.3%) and right otolith (13.8%) readings was high for sectioned otoliths. Again this was thought to be due to inexperience with reading otoliths with this new method. There was no major bias between readers, however. Any bias that existed was between $\pm$ 0.75 years.

Only age readings from the primary reader showed that ages estimated from right otoliths were significantly lower than ages estimated from left otoliths, and this tended to be at older ages ($p < 0.001$; Figure 3). This was similar to what was seen in whole otoliths, except both primary and secondary age readers tended to underage the right otolith. A paired comparison of sectioned otoliths and whole otoliths revealed strong bias after age 7 in right otoliths and age 8 in left otoliths (Figure 4). Some bias existed between whole and sectioned otoliths for the primary ager when ageing the right otolith for the first three years, but the bias was small (less than 1 year) and is likely due to misinterpretation of the first annulus. Age estimates from whole otoliths were significantly lower than ages estimated from sections ($p < 0.001$), and in some cases sections gave estimates 6 years older than estimates from whole otoliths.

The photographs in Figures 5-7 show some common features of yellowtail flounder sections, including prominent settling checks and clear annuli. Spawning checks are seen, but not consistently. In some cases the first years show a split in each annuli. Pictures were taken with Optimas 6.2 at a magnification of 10X.

Part II Length Frequency Analysis

Pelagic 0 group fish

In the ichthyoplankton surveys examined, yellowtail flounder larvae were found in the range of 3 mm to about 40 mm in length. The 1986 to 1988 data, from Frank *et al*. (1992) (Figure 8), shows that 0-group larvae are present in the range of 4-35 mm in September on the southern Grand Banks, with an average size of about 19.5 mm. The range in size may be due to a prolonged spawning season. Data from 1994-99 annual ichthyoplankton survey (Figure 9) show the same trends but numbers are too low for this data to be valuable. They do indicate, however, that there may be a bimodal distribution, with a cluster of fish sizes under 4 mm in length (perhaps newly hatched) and a number of fish between 16 and 40 mm. This may be due to peak spawning times, or some environmental variable, influencing size at metamorphosis.

Overall, the data indicates that there are probably no pelagic yellowtail larvae present above 40 mm (4 cm) and these can be confidently regarded as young-of-the-year or 0 group fish.

Demersal 0 group fish

In the juvenile groundfish surveys, the peak at 4 cm, representing what is probably the demersal 0 group fish, is distinguished in only the males for the 1987 data (Figure 10), when the survey was done late in the year (November); presumably the fish were newly settled. Obviously fish begin to settle between 2-4 cm in length, with some variation, depending on a number of factors. Because these fish are not present in September, it gives further validity to the fact that these are 0 group fish. The average length of demersal 0 group yellowtail is approximately 3 cm.

Length-frequency analysis from juvenile surveys - males

It can be stated fairly confidently that male fish present between 5 and 10 cm in the fall of the year are age 1 fish (Figure 10). That is, these fish were hatched in the summer of the previous year. These fish have an average length of 7 cm. Following from that, a 2-year old male fish would correspond to the mode that falls between 10 and 15 cm in length, with an average of 12.2 cm. Ages three to five were difficult to distinguish, and ages beyond five impossible to separate. However, following strong year classes enables the estimation of ages up to age 5, see the strong 1985 year-class at age 1 in 1986 in Figure 10.

Length-frequency analysis from juvenile surveys - females

In Figure 11, modal peaks representing ages 1 and 2 were quite distinct and could be separated easily. There were no 0-group females found in any year. This may be because no otoliths were taken from female fish less than 10 cm in the 1987 survey. Age 1 fish had an average length of 7.4 cm, and age 2 fish had an average length of 13.9 cm. These sizes are slightly larger than the same aged male fish. Similar to males, peaks for ages three and up are difficult to distinguish.

Length-frequency analysis from annual fall surveys - males

Fall surveys tended to exclude younger age groups, and it was difficult to distinguish most peaks. Estimates are provided in Figure 12. It is interesting that the average length provided by peaks 1 and 2 (presumably ages 1 and 2) are slightly higher than values from the juvenile survey. This is probably due to the fact that peaks were not as distinct as those seen in the juvenile surveys which may be related to differences in size selectivity.

Length-frequency analysis from annual fall surveys - females

Again, females tended to be somewhat larger than their male counterparts (Figure 13). The same problems were encountered with the female fall data as the male fall data; that is, the peaks were hard to discern and thus average length values may be erroneous.

Discussion

At the NAFC, yellowtail flounder are aged using whole otoliths that may be ground to increase the visibility of annuli. This study showed that there is no statistical difference between ages estimated by ground or unground whole otoliths. This study also allowed for the direct comparison of ageing using the 'standard' method of ageing whole otoliths and a 'new' method' of using thin-sectioned otoliths for age determination.

There was also a significant difference between ages derived from reading left and right otoliths in both whole and sectioned yellowtail otoliths. This is probably due to the asymmetric nature of flatfish otoliths, in that the dorsal, or right, otolith usually is constricted and thickened relative to the left otolith. This is thought to be result of the influence of body rotation in flatfish (Hunt, 1992). Thus, on the surface, annuli are harder to distinguish, and ages are underestimated at older ages, and internally, sections appear compressed and asymmetrical, and are also more difficult to discern. Age validation is needed to determine which otolith provides a more accurate age, but it is likely that the left is more accurate due to the nature of otolith placement inside the flatfish head.

The traditional method of ageing yellowtail flounder from the Grand Banks (Division 3LNO) appears to underestimate the ages of these fish. It is obvious from age bias graphs that the problem with the present ageing technique appears after age 7. Ages estimated from thin-sections indicate that male yellowtail flounder may live up to 13 years old and females up to 16 years old. However, caution must be exercised, because there has been no direct age validation for this method in yellowtail flounder, other than length-frequency analysis, which can only confirm the earliest ages. Length frequency validates younger ages given from whole otolith readings, which do not differ from otolith section readings up to this age. Thus, the results supports the suggestion that whole otoliths are suitable for ageing yellowtail flounder at least up to age of 7.

Age validation is undoubtedly the most important part of the age determination process. There have been criticisms about other studies in that age validation studies have not been completed on either whole otoliths or sections in order to determine whether an annuli actually represents a year of growth (see for example Beamish and McFarlane, 1983). Although it is attempted for younger fish through length-frequency analysis and can be done for mid-age fish through marginal increment analysis, it is especially difficult to do for old fish. Incorrect interpretations of annuli may result in serious ageing errors, which lead to a misunderstanding of the dynamics of a fish population. Errors in ageing may result in an accumulation of estimates in the age at which the ageing technique becomes ineffective. Such may be the case for yellowtail flounder. This may cause growth rates to appear falsely inflated, and overestimate natural mortality in a stock (Beamish and McFarlane, 1983). Therefore, age validation is required, whereby it is demonstrated that the amount of rings present on an ageing structure is roughly equal to the age of the fish. Validation methods are needed to confirm the ageing results for yellowtail flounder presented here in older ages of yellowtail flounder. The next step in this validation process is to attempt marginal increment analysis. Marginal increment analysis works usually for mid-aged fish, because the edge type is hard to distinguish in the older fish, in which annuli are laid down close together. However, this method may not be valid for all ages.

Although we cannot say with certainty that ages from sectioned otoliths are accurate, underageing by whole otolith reading in older fish has been shown in many species of fish, and ageing using internal annuli has been validated in a vast number of species. For example, ages estimated from internal annuli have been validated by direct methods such as chemical marking by 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). In redfish (*Sebastes mentella*) (Campana *et al*., 1990), and Pacific grenadier (*Coryphaenoides acrolepis*) (Andrews *et al*., 1999), the ageing method 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 (*Melanogrammus aeglefinus*) (Campana, 1997) and southern bluefin tuna (*Thunnus maccoyii*) (Kalish *et al*., 1996) by using assays of bomb radiocarbon content with accelerator mass spectrometry. Thus, we have reason to believe that ages from sectioned otoliths are probably more reliable then those from the traditional whole otolith method used to age Grand Bank yellowtail flounder.

In summary, this paper provides supporting evidence that the standard ageing technique of reading whole otoliths is not suitable for fish beyond age 7. Sectioning the otoliths offers more promise for ageing older fish. However, age validation of otoliths must be carried out to confirm our ageing of fish with the sectioning technique.

Conclusions & Recommendations – the Next Steps

1. Conduct ageing of yellowtail flounder with scales. A collection of 204 sets of scales have been taken from every fish from which otoliths were used in this study. It is expected that scales will be adequate for ageing yellowtail flounder up to about 7 years, and can be used to validate age together with length-frequency analysis.
2. Conduct marginal increment analysis on the 'new' method of thin-sectioning of otoliths and compare the results with the 'standard' method. This may validate the 'new' method for ages up to possibly 9 years old.
3. For a selection of large (presumably old) fish from the NAFC archived otolith collection, carry out assays of bomb radiocarbon content to validate ageing of yellowtail flounder past age 7, using the 'new' method of thin sections for age determination. This method is considered to be the most accurate and feasible method currently available for validating ageing in old fish.
4. Organize a workshop on age determination of yellowtail flounder. The focus would be on aging techniques (otoliths, scales), validation methods, quality control (precision and agreement among age readers), and otolith exchange programs. Candidates to attend this workshop are Canadian and United States laboratories.

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Estimated Ages Between Left and Right Whole Otoliths

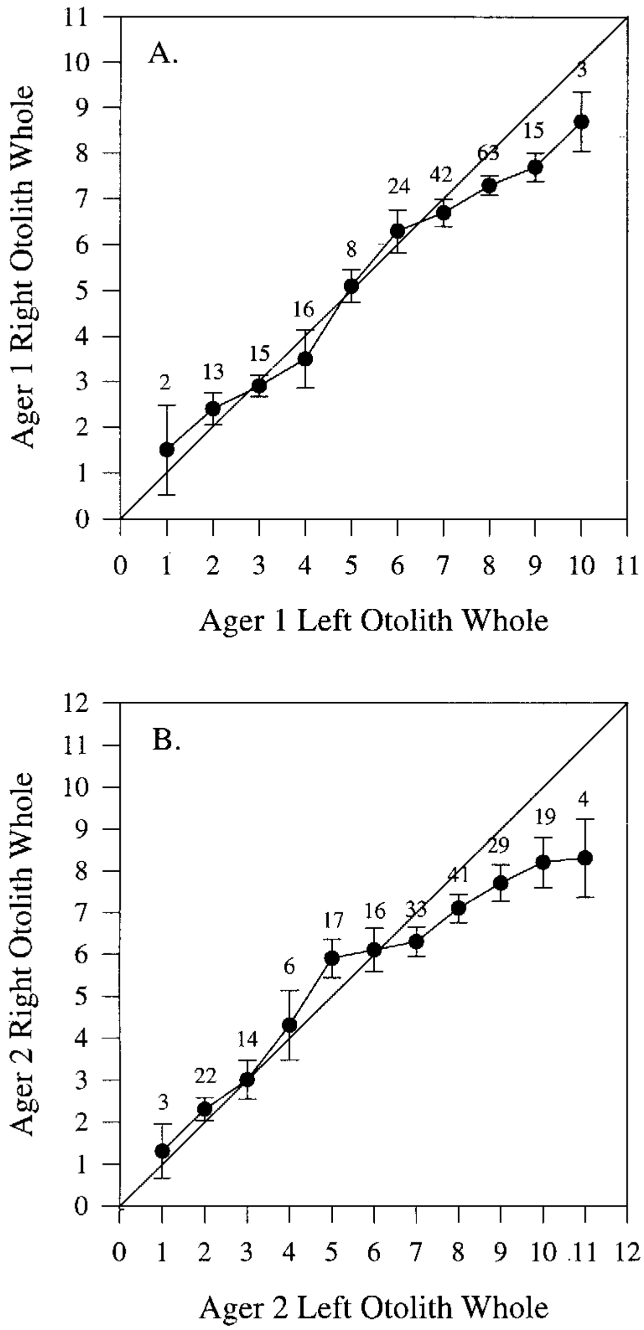

Figure 1. Age bias graphs for the primary ager (ager 1) (A) and secondary (Ager2) (B) for left and right whole otoliths. Each error bar represents the 95% confidence interval about the mean age assigned for one otolith for all fish assigned a given age for the second otolith. The 1:1 equivalence (solid line) is also indicated. Numbers plotted with symbols are the sample size at each age.

Estimates of Age From Ground versus Unground Otoliths

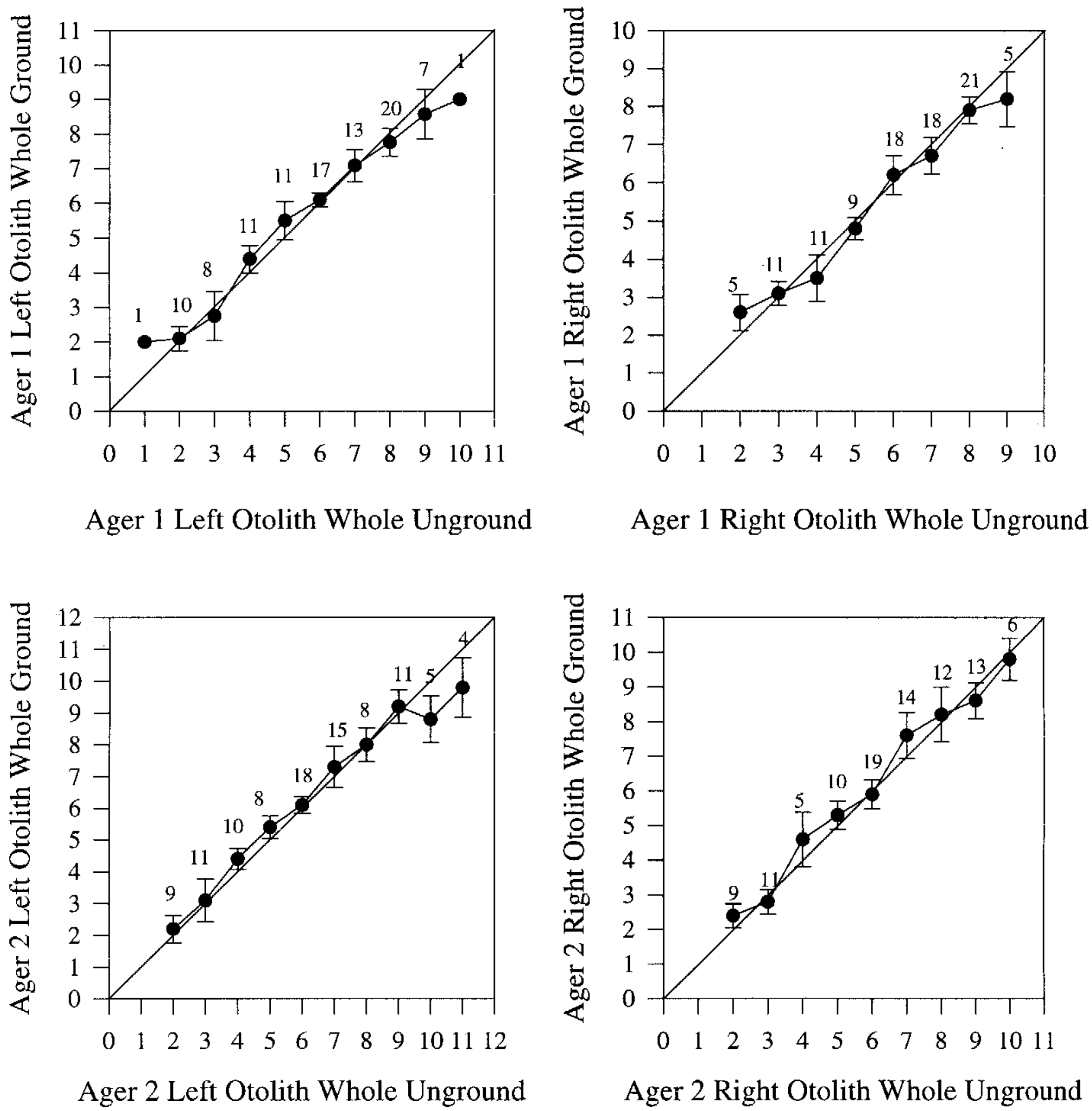

Figure 2. Age bias graphs for left and right ground versus unground otoliths. See Figure 1 for details.

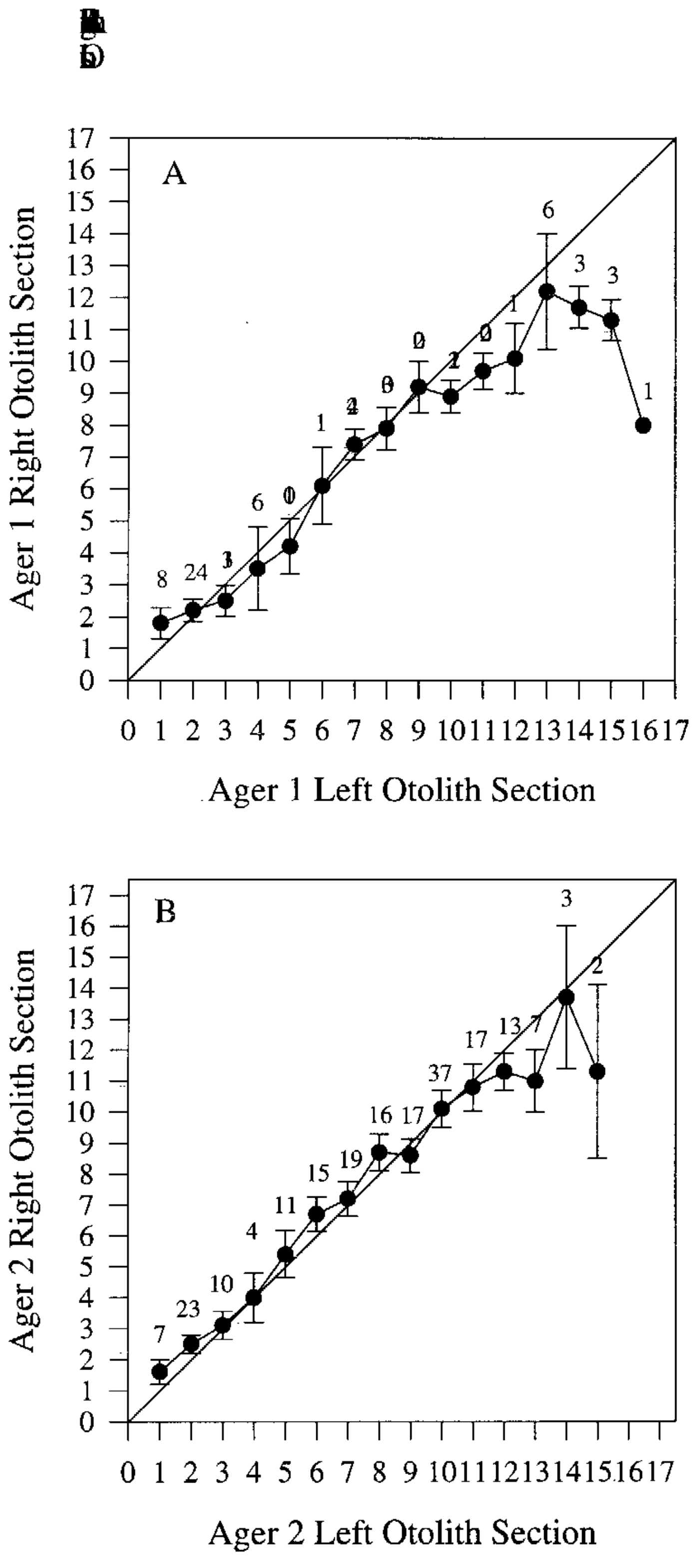

Figure 3. Age bias graphs for primary (ager 1) (A) and secondary (Ager 2) (B) ager for left and right sectioned otoliths. See Figure 1 for details.

Estimated Ages Between Whole and Sectioned Otoliths

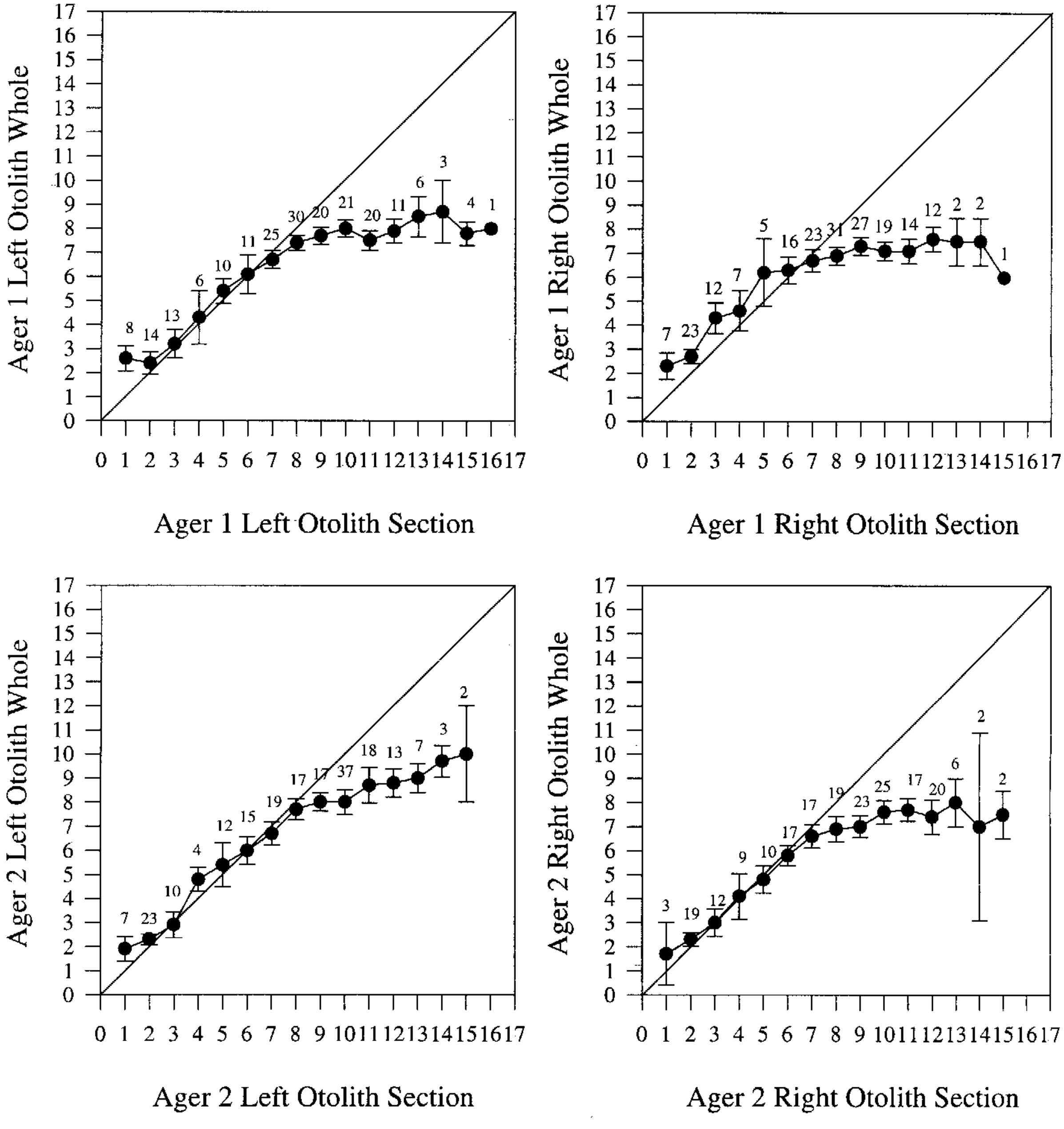

Figure 4. Age bias graphs for left and right whole versus sectioned otoliths. See Figure 1 for details. The departure from the 1:1 equivalence line indicates signifcance at $\alpha = 0.05$.

Figure 5
Otolith section from a 14 cm age 2+ female yellowtail flounder showing a settling check and two annuli.

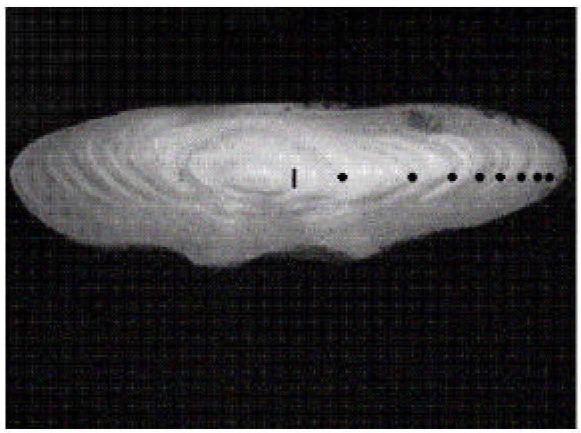

Figure 6
Otolith section from a 33 cm age 8 male yellowtail flounder showing a settling check and well defined annuli 1-8.

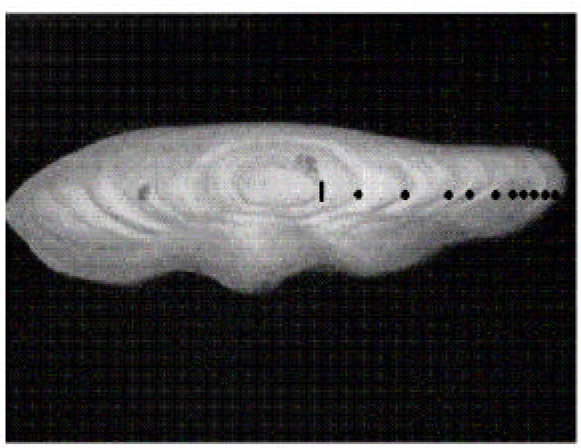

Figure 7
Otolith section from a 40 cm age 10 male yellowtail flounder showing a settling check and well defined annuli 1-6 and poorly defined annuli 7-10.

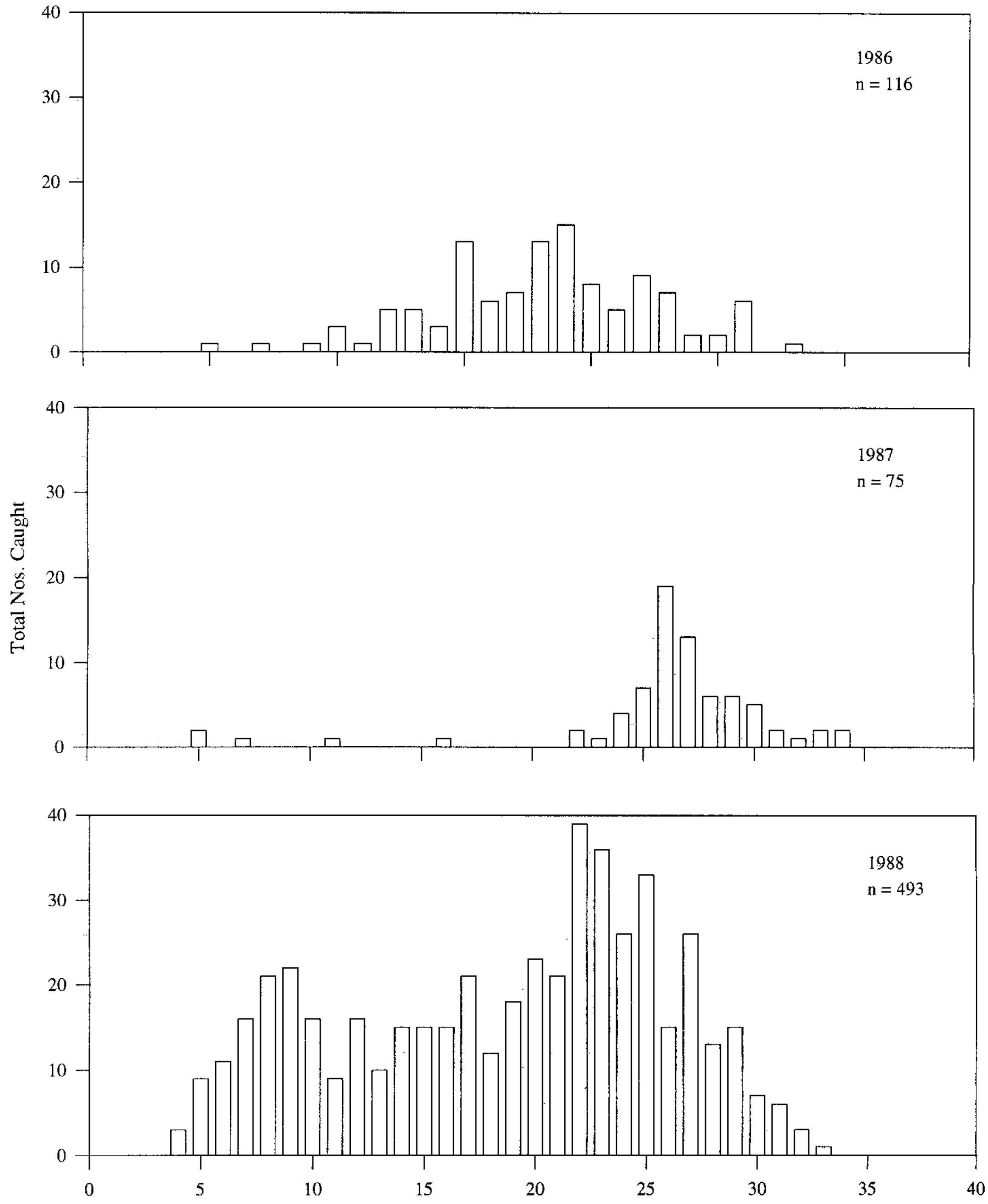

Figure 8 Length compositions (mm) of pelagic yellowtail flounder larvae from surveys conducted on the southern Grand Banks during September for 1986-1988 (n = total number caught). From Frank *et al.* (1992), used with permission of J. Carscadden, DFO, NF.

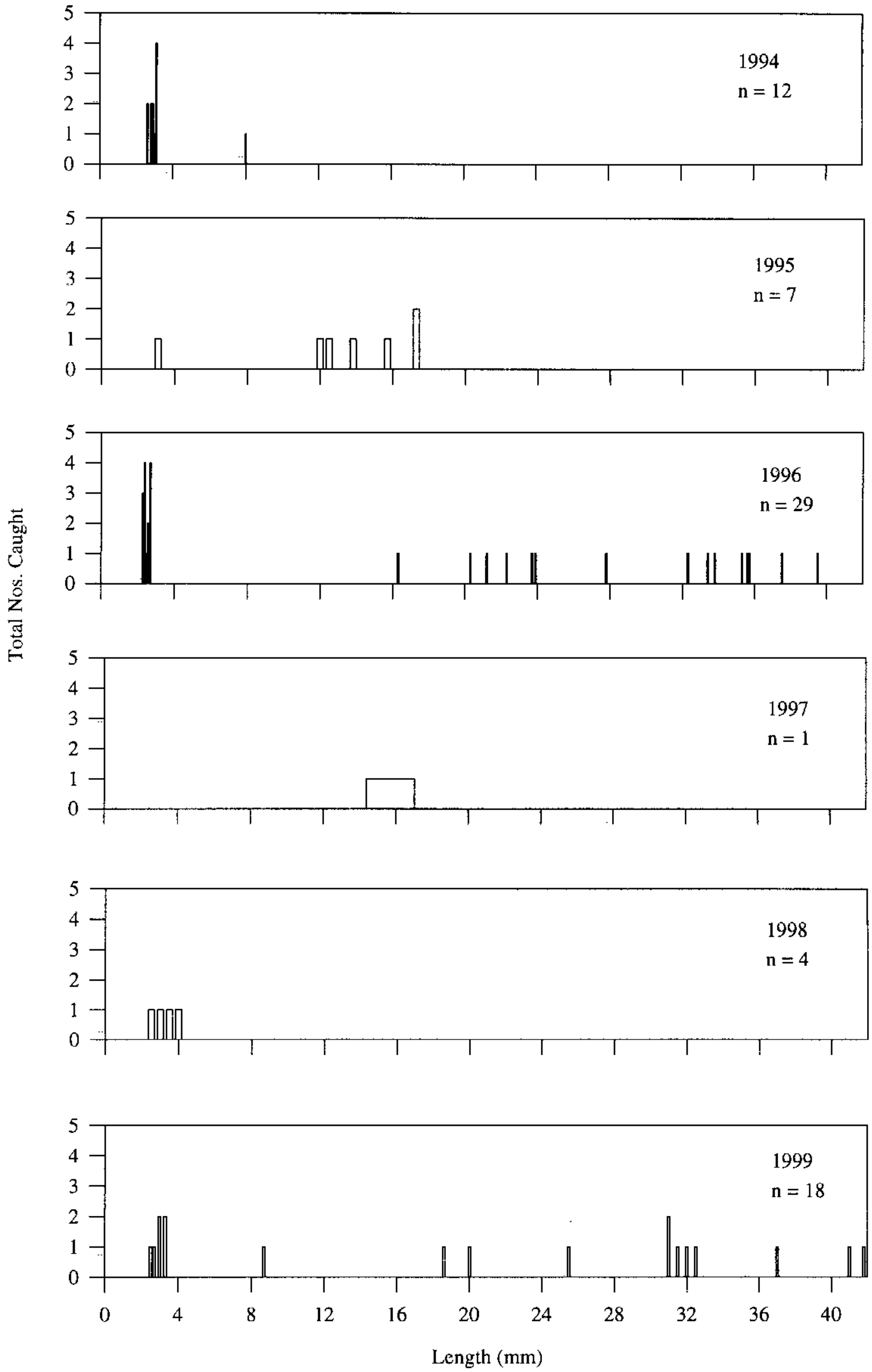

Figure 9 Length compositions (mm) of pelagic yellowtail flounder larvae from annual pelagic 0 group surveys on the southern Grand Banks during late August 1994-1999 (n = total number caught). Used with permission of John Anderson, DFO, NF.

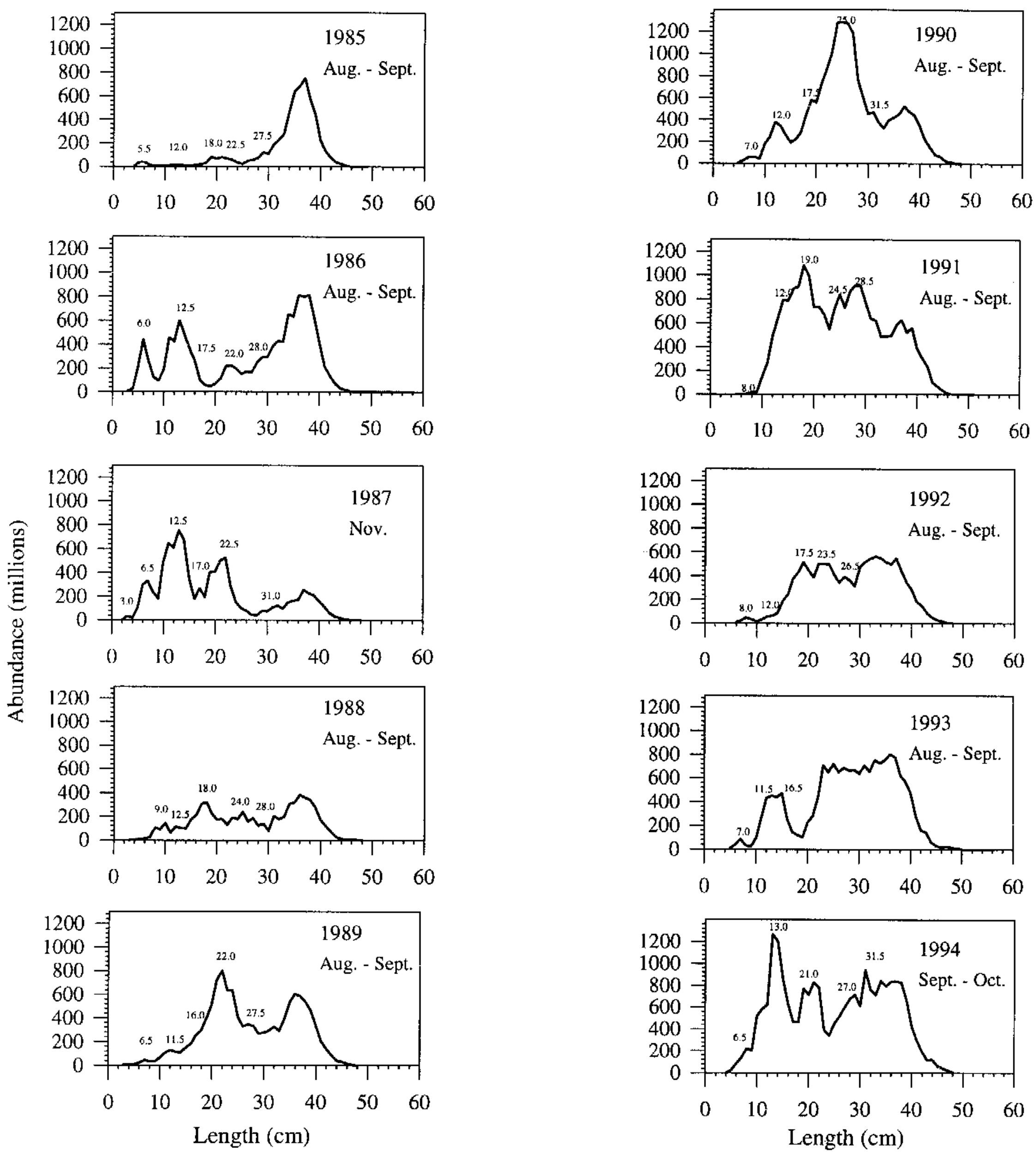

Figure 10 Length compositions (cm) of male yellowtail flounder from annual DFO juvenile groundfish surveys from 1985-1994. Time of year is indicated. Numbers are average length of modes.

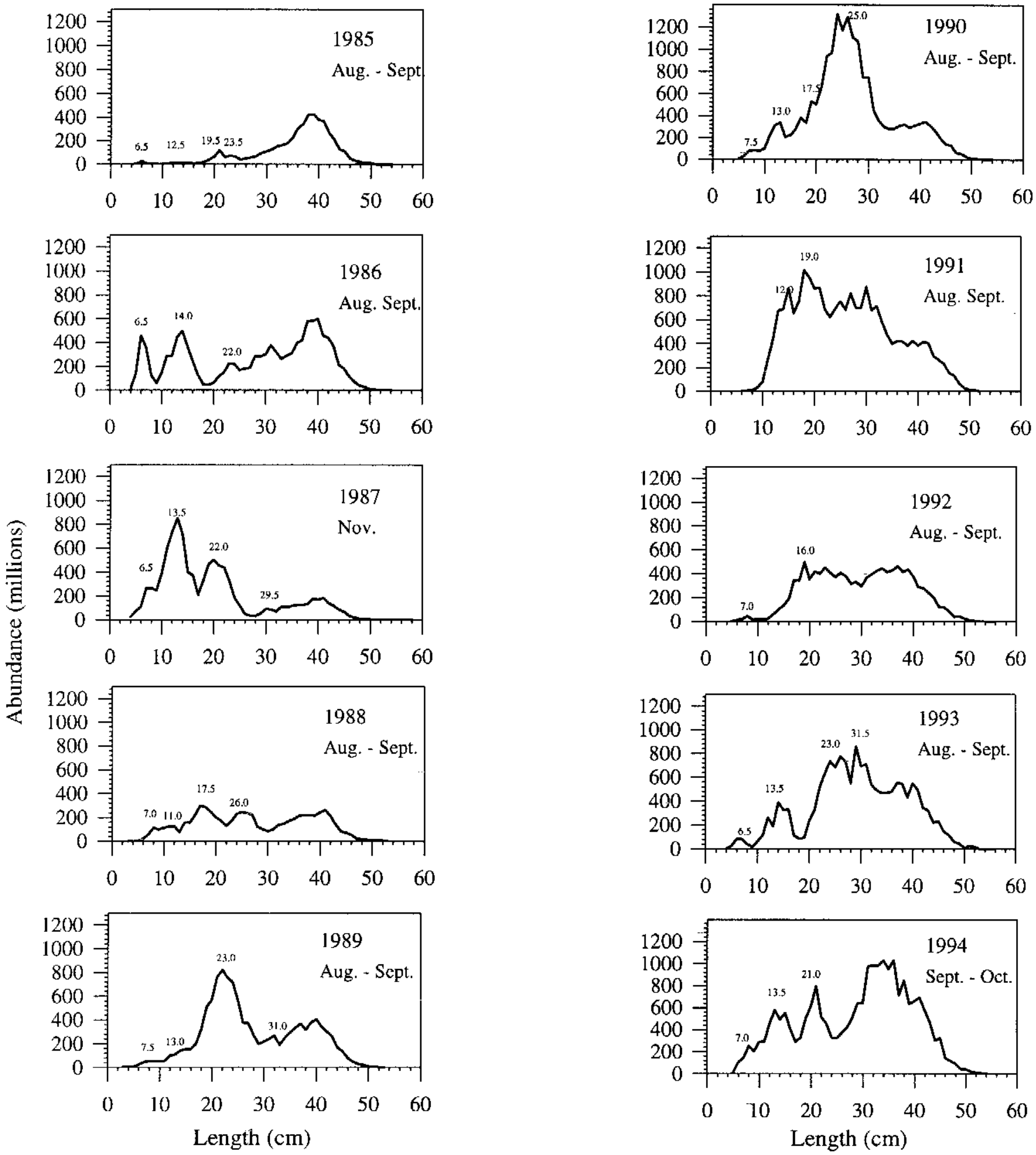

Figure 11 Length compositions (cm) of female yellowtail flounder from annual juvenile groundfish surveys from 1985-1994. Time of year is indicated. Numbers are average lengths of modes.

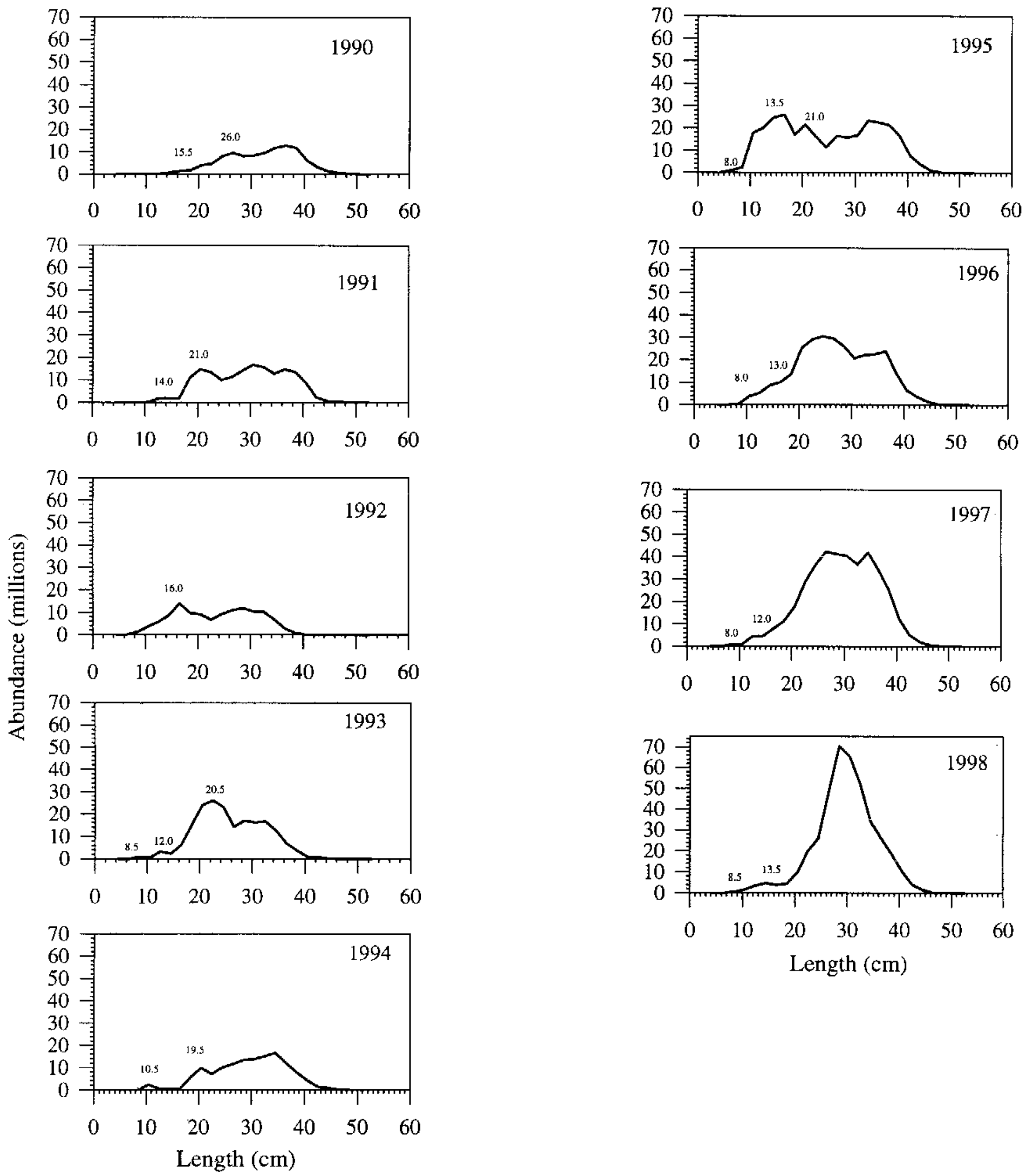

Figure 12 Length compositions (cm) of male yellowtail flounder from annual DFO fall surveys from 1990-1998.

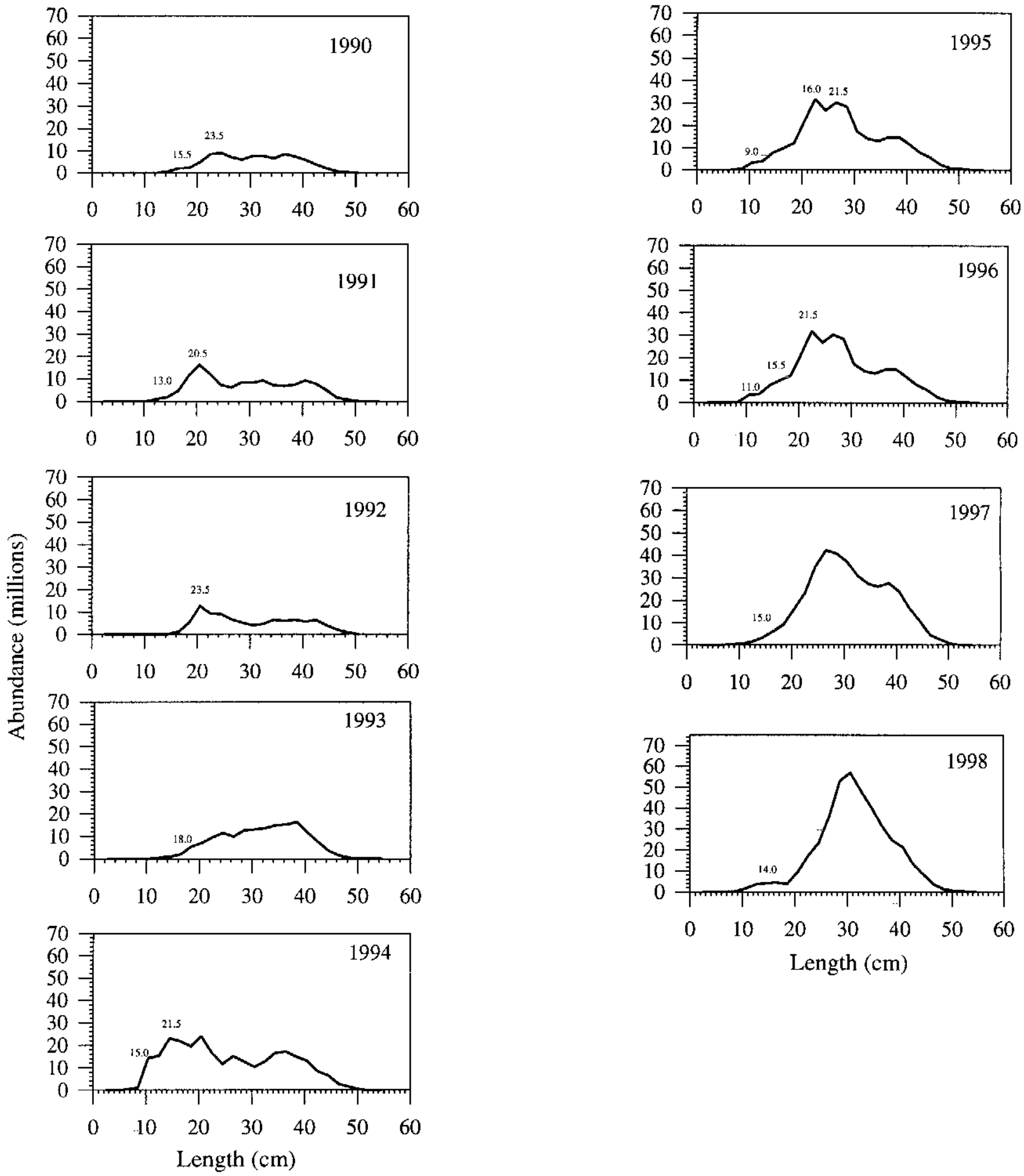

Figure 13 Length compositions (cm) of female yellowtail flounder from annual DFO fall surveys from 1990-1998.