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Age and Growth of Yellowtail Flounder in NAFO Divisions 3LNO: An Update

by

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**Abstract**

The ability to perform age determination based on the growth rings on hard structures of fish is important to fisheries management. Estimates of yellowtail flounder growth rate, age at maturity and longevity are required for stock assessment purposes. In a study by Whalen *et al.* (2000), the traditional method of reading yellowtail flounder otoliths whole (surface) was found to fail at the age of seven; after which, it was found to be more accurate to use thin sections to age fish. We continue these investigations using baked thin sections, and scales, and compare these new findings with the previous methods to assess their contribution to accuracy of ageing yellowtail flounder. We also investigate growth by using new age bias plots with an increase in sample size and age range.

**Introduction**

Age determination in fisheries is vital to the successful management of a fish stock. It enables scientists to establish the age composition of a population of fish, and thereby make decisions based on mortality, recruitment and assign catch quotas accordingly. In addition, knowledge of the longevity of a stock of fish can add to the pool of ecological information regarding the species. In temperate climates, most ageing is done through the use of hard parts such as otoliths or scales. There are many methods used to enhance the annuli of these hard parts, and by comparing age estimations from each, one can determine whether there is a problem ageing with a current technique and determine which is the preferred ageing method. However, 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. This is a time-consuming and expensive process, and one that may be extremely difficult for older, long-lived fish.

In 2000, studies were carried out to determine whether the traditional method of ageing yellowtail flounder was suitable and comparable to thin-sectioning. This paper continues these investigations by looking at baked thin sections, a technique known to enhance the visibility of annuli in some fish, and scales. Growth curves of males and females are constructed from ages estimated from thin-sectioning.

**Materials and Methods**

Methods for the preparation and reading of whole and sectioned otoliths follow those of Whalen *et al.*, 2000. Approximately 200 otoliths were added to increase sample size and age range to the comparisons of readers and methods. Only the left otolith was read, as this otolith is the most symmetrical and therefore easiest to read (Whalen *et al.*, 2000; Hunt, 1992). Readings were compared using coefficient of variation (CV) to test for precision, age bias plots were used to test for bias and t-tests were also used to compare differences statistically (Campana *et al.*, 1995; Walsh and Burnett, 2001).

### Embedding scales

Scales were removed from fish by scraping a knife along the caudal peduncle of the dorsal or eyed side of the fish. The knife blade is then placed between paper and the adhering scales removed onto the paper.

Scale impressions are prepared by placing 5-8 scales sculptured side down on a 0.2 mm thick piece of cellulose acetate plastic. This plastic is placed on a warming area (above a lamp) of a jeweler's press and when sufficiently warm, is rolled through the press. The press embeds the shape of the scale into the plastic and when removed, can be stored indefinitely in a dry envelope. If the scales are very small, they can be sprinkled on the plastic and then rolled.

Scales were read using a microfiche reader at about 25 X by both readers separately and then compared. Three hundred and twelve (312) samples were examined by the readers.

### Baking sections

The left otolith of each sample was sectioned using a Buehler Isomet low-speed saw and two diamond-tipped blades running simultaneously. A transverse section, approximately 0.3 mm thick was removed and read, immersed in alcohol, under reflected light, at a magnification of 25-40X. Both readers aged the section without consultation. Afterwards, the sections were placed on an aluminum plate, which contained separate wells, and then baked in a Thermolyne muffle furnace at 400°C for 1 ½ to 2 minutes. Baked sections were then removed and read in the same manner, by each reader, as before. Ninety-three (93) sections were examined with this approach.

### Growth curves

Von Bertalanffy growth curves, based on length-at-age from section readings, were fitted separately for males and females by non-linear regression:

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

where  $l_t$  = the length at age  $t$  and  $L_\infty$ ,  $K$  and  $t_0$  are the growth parameters.

This was done using R script.

## **Results and Discussion**

### Whole Otoliths versus Sectioned Otoliths

When sectioned otoliths were read and compared, there was no bias seen between readers for the left otolith. The CV between readers was high (11.5%) for sectioned otolith readings, which may be due to the fact that both readers are relatively inexperienced reading otoliths in this manner. The t-test revealed no significant difference between readers ( $p = 0.9151$ ).

A comparison between thin sections and otoliths (Figure 1) revealed extreme bias between the two methods for both readers, as was seen in Whalen *et al.* (2000). Generally, after age 7, ages derived from whole otoliths tend to be much lower than those estimated from sections. There was also a tendency to bias downward younger ages in thin sections and this is thought to be due to a mis-interpretation of the first annulus. Recent evidence (Dwyer *et al.*, 2001) indicates that the bias at older ages between whole and sectioned otoliths is actually more extreme than these age bias plots show, and that whole otoliths are unsuitable for ageing yellowtail flounder over the age of 7.

### Baked Sections versus Unbaked Sections

There were no differences between readers for baked section age estimates (t-test:  $p = 0.7768$ ). The CV was 8.2%, which was lower than for unbaked sections. There was a significant difference between baked and unbaked sections for Reader 1 (CV: 12.2%; t-test:  $p = 0.0015$ ) but not Reader 2 (CV: 6.3%; t-test:  $p = 0.2214$ ) (see Figure 2); however, the bias for Reader 1 was not obvious (less than a year with a small samples size) and deemed negligible.

Baking sections is thought to enhance the annuli by making them a caramel colour. However, it was concluded that there was no advantage in baking the thin sections for ageing yellowtail flounder.

### Scales versus Sections

Readers found scales difficult to read, as neither reader had much experience ageing them. There was a significant difference ( $p = 0.0005$ ) in age estimation between readers, with Reader 2 tending to underage the oldest ages. The CV was high, at 12.4% but again bias was negligible and less than a year.

Both readers showed bias after age 7 (departure from the 1:1 equivalency line) when comparing sectioned otoliths and scales, with both readers seeing fewer annuli in scales than in sections (see Figure 3). Again, there seemed to be a problem interpreting the first annulus.

### Growth curves

Males tended to grow more slowly than females (Figures 4 and 5), which has been reported in the literature (Pitt, 1974). However, the parameters of the von Bertalanffy curve are quite different than other authors have reported (Pitt, 1974; Walsh and Morgan, 1999). These parameters are listed in Table 1 and show that growth is slower than has been reported, but the growth rate is above that which has been reported for fish tagged in the early 1990s (Walsh and Morgan, 1999). The slow growth rate in the tagging study may be attributed to fish being tagged at a time when water temperatures were extremely low (Colbourne, 1993). We feel that the growth curves generated from ages estimated from thin sections are a more accurate representation of growth of yellowtail flounder. However, it is important to note that the misinterpretation of the first annulus may cause a change in the growth curves. We therefore also include growth curve parameters in which we assume we are off by one year in the younger ages.

Research has revealed that whole otoliths or scales tend to underestimate fish age (Beamish and McFarlane, 1995). This is also the case for yellowtail flounder, as fish aged with whole otoliths give a maximum age of 10 years, those aged with scales give a maximum age of 14 years and those aged with sections give a maximum age of 16 years in this study. Dwyer *et al.* (2001), using tagging returns and bomb radiocarbon assays, validated thin sections as giving the most accurate ages, but even misinterpretation of the annuli in the oldest fish using this method can underestimate the true age of the fish. Yellowtail flounder on the Grand Banks may reach a maximum age of >25 years old. However, it can be said with certainty that annuli read from the internal surface of the otolith are the most accurate, and this has been validated for English sole (*Parophrys vetulus*) (McLellan and Fargo, 1995); redfish (*Sebastes mentella*) (Campana *et al.*, 1990); haddock (*Melanogrammus aeglefinus*) (Campana, 1997). For yellowtail flounder, either scales or whole otoliths may be used and preparation is less labour-intensive than thin sections for fish up to age 7. In order to decrease the CV between readers, especially for thin sections, it is recommended further that readers draft a set of protocols for reading thin sections, and the first annulus should be validated to decrease bias in younger fish. The problems with the first annulus will affect the growth curve parameters but not the final shape of the curves.

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Table 1. Summary of parameters of von Bertalanffy growth equation for yellowtail flounder from different studies.

Location	Sex	Ages fitted	$L_{\infty}$	K	$t_0$	Reference
Grand Bank	M	3 to 11	42.07	0.41	1.39	Pitt, 1974
Grand Bank	F	3 to 12	48.12	0.29	0.80	Pitt, 1974
New England	M + F	2 to 7	50.00	0.34	-0.26	Lux and Nichy, 1969
St. Pierre Bank	M	2 to 8	48.38	0.15	0.50	Berthome, 1976
St. Pierre Bank	F	2 to 9	56.44	0.13	0.50	Berthome, 1976
Grand Bank	M	1 to 16	53.44	0.17	0.07	This study
Grand Bank	F	1 to 25	56.33	0.17	0.20	This study

Table 2. Summary of parameters of von Bertalanffy growth equation for yellowtail flounder from this study corrected for possible first annulus misinterpretation.

Location	Sex	Ages fitted	$L_{\infty}$	K	$t_0$	Reference
Grand Bank	M	2 to 16	58.99	0.14	0.59	This study
Grand Bank	F	2 to 25	57.63	0.16	0.88	This study

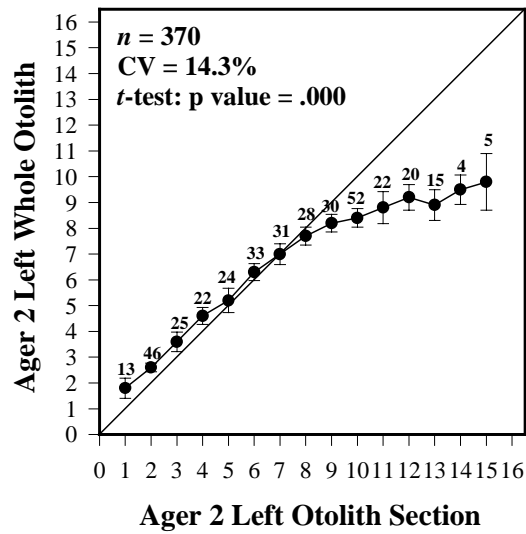
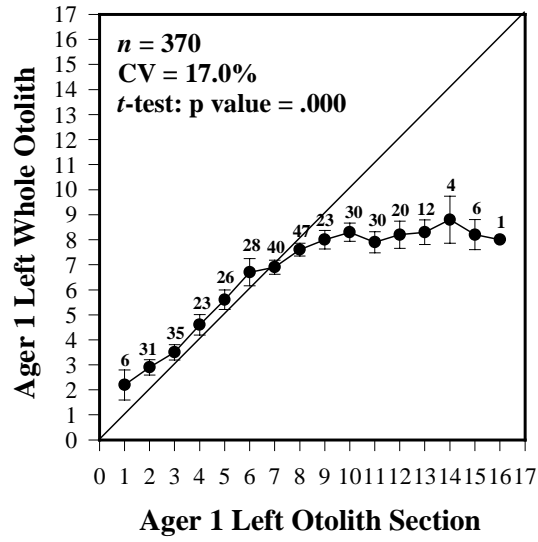


Fig. 1. Age bias plot for agers 1 and 2 whole otoliths and sectioned otoliths. Each error bar represents the 95% confidence interval about the mean age assigned for one otolith for all fish assigned a given age for the second otolith. The 1:1 equivalence (solid line) is also indicated. Numbers plotted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

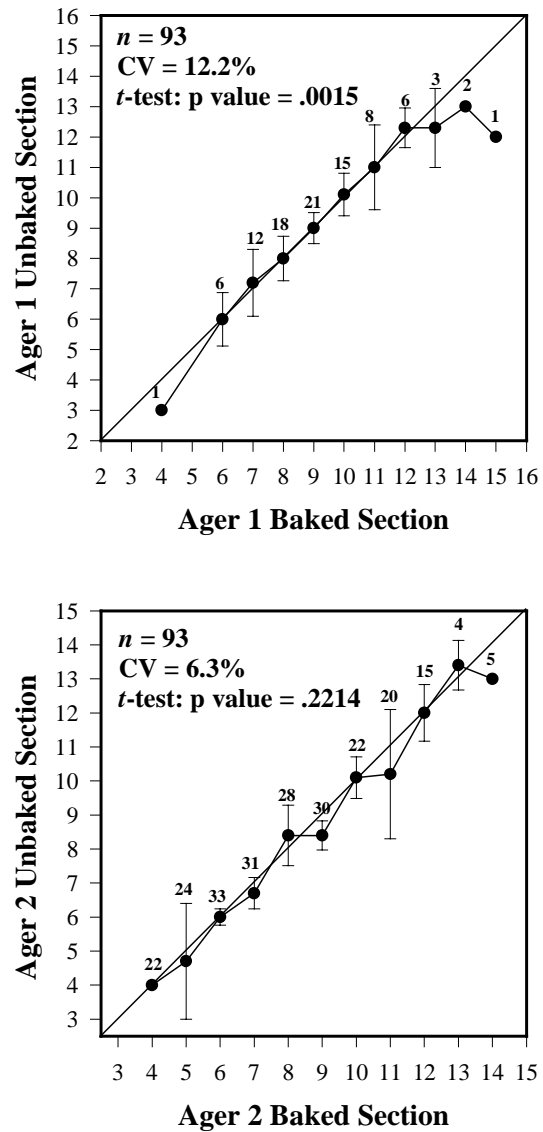


Fig. 2. Age bias plot for agers 1 and 2 baked and unbaked sectioned otoliths. Each error bar represents the 95% confidence interval about the mean age assigned for one otolith for all fish assigned a given age for the second otolith. The 1:1 equivalence (solid line) is also indicated. Numbers plotted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

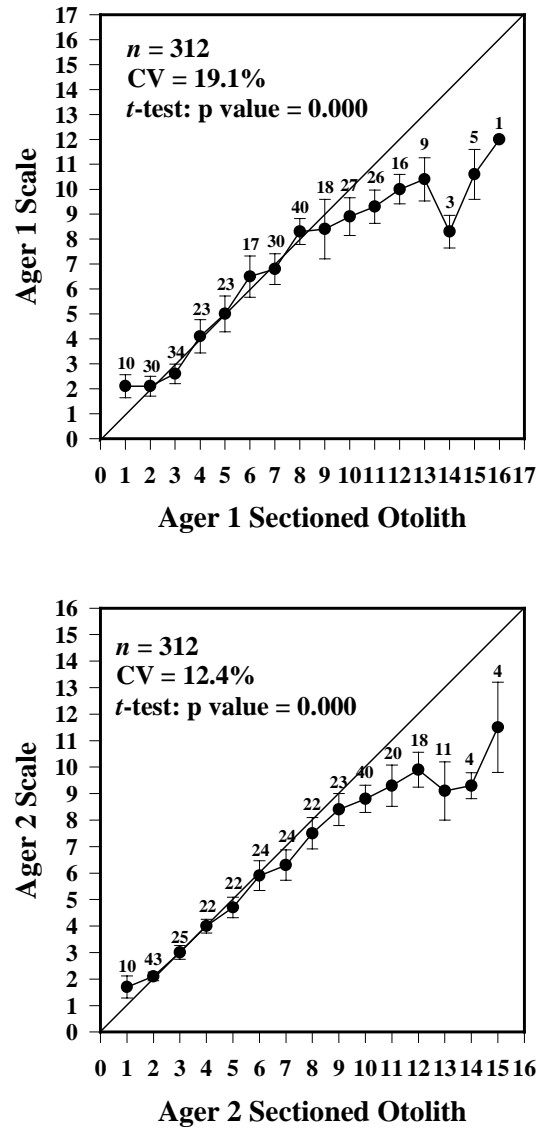


Fig. 3. Age bias plot for agers 1 and 2 scales and sectioned otoliths. Each error bar represents the 95% confidence interval about the mean age assigned for one otolith for all fish assigned a given age for the second otolith. The 1:1 equivalence (solid line) is also indicated. Numbers plotted with symbols are the sample size at each age.

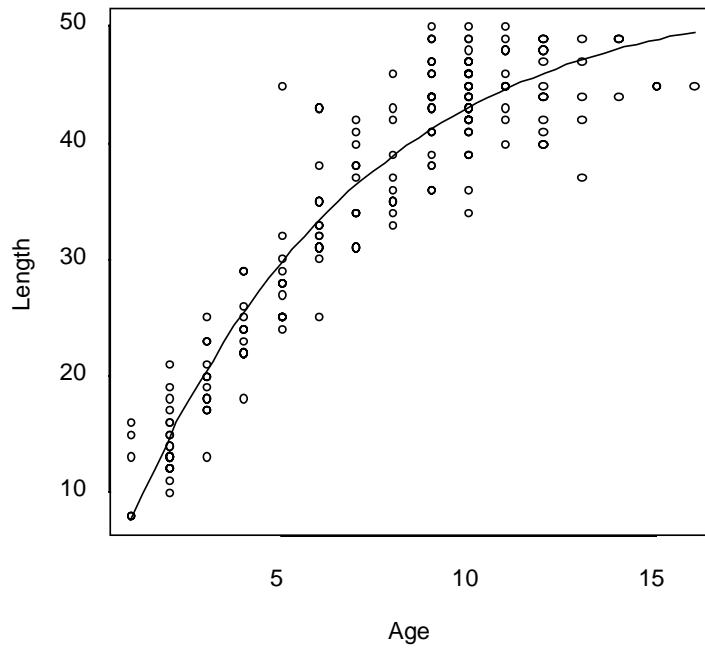


Fig. 4. Von Bertalanffy growth curves fitted to length at age data of male yellowtail flounder.

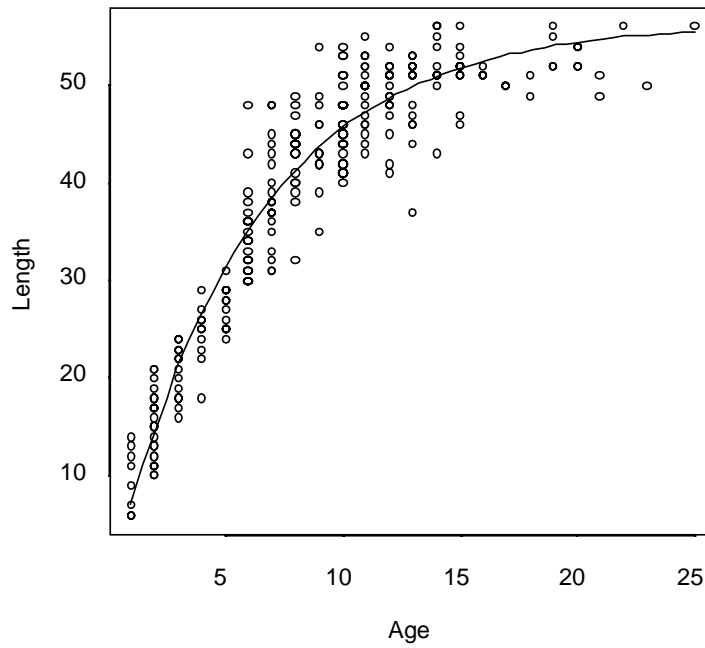


Fig. 5. Von Bertalanffy growth curves fitted to length-at-age data of female yellowtail flounder.