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Use of backcalculations on NAFO Subdivision 5Ze cod otoliths for the correct assignment of settling checks and first annuli

by

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ABSTRACT

The purpose of this study was to initiate investigation on topics discussed at the 1991 Georges Bank Cod and Haddock Ageing Workshop, and on other issues directly related to the ageing of 5Ze cod. Discrepancies between the Canadian and USA catch-at-age data have been of concern in the past, and improper ageing has been suggested by USA age readers as a possible cause. The possibility of misinterpretation of otolith features such as larval settling checks and subsequent incorrect age assignments was the main objective in this study. Whole larval otoliths and sectioned 1+ otoliths from commercial samples and groundfish surveys were used to measure settling checks, first annulus and second annulus cross-sectional widths.

The Image Analysis System facilitated the study by objectively quantifying check and annuli sizes. Settling check XS width averaged <1 mm, first annulus XS width ranged between 2 and 2.5 mm, and second annulus XS width averaged about 4.5 mm. The methods proved to be of practical application for verification of age interpretations. Similar measurements should be done for each species and stock.

RÉSUMÉ

La présente étude visait à analyser les discussions amorcées à l'atelier sur l'établissement de l'âge de l'aiglefin et de la morue du banc Georges, tenu en 1991, et d'autres questions directement reliées au calcul de l'âge de la morue de 5Ze. Des écarts entre les données sur les prises selon l'âge compilées au Canada et celles qui proviennent des États-Unis ont été sources de préoccupation par le passé, et les spécialistes américains du calcul de l'âge ont suggéré qu'ils pouvaient être dus à des erreurs dans la lecture de l'âge. La possibilité d'une interprétation erronée des caractéristiques des otolithes, comme les marques d'implantation des larves et le calcul des âges subséquents était le but premier de l'étude. Des otolithes entiers de larves et des otolithes sectionnés d'âge 1 + provenant d'échantillons commerciaux et de campagnes d'évaluation du poisson de fond ont servi à mesurer les largeurs transversales des marques d'implantation des larves ainsi que du premier et du deuxième anneaux.

Le système d'analyse d'image a facilité l'étude en question en quantifiant objectivement la taille des marques d'implantation des larves et des anneaux. La largeur transversale des marques d'implantation s'établissait en moyenne à < 1 mm, celle du premier anneau à 2-2,5 mm et celle du deuxième anneau en moyenne à 4,5 mm. Cette méthodologie s'est avérée utile à la vérification pratique de l'interprétation des âges. Des mesures semblables devraient être prises pour chaque espèce et pour chaque stock.

INTRODUCTION

Errors or misinterpretations in ageing directly affect the catch-at-age data and subsequent stock assessment, therefore otolith characteristics used for ageing must be interpreted in a precise manner. The age reader requires an unbiased and precise method of choosing a good section, identifying otolith characteristics, and verifying ages. Precision provides age assignments which are reproducible by the age reader. Verification of correctness of these ages, or accuracy, should still be done by back calculations, tagging or growth experiments. The sectioning procedure itself can cause misinterpretations which could be avoided if sufficient information on annuli characteristics were available.

Many annuli patterns are accepted by consensus, based on an age reader's degree of experience. These interpretations can be evaluated and confirmed if unbiased, quantitative data are obtained. For example, a 1991 Canada/USA Ageing Workshop held at St. Andrews, N.B. concluded that a common characteristic of Georges Bank cod, a "settling check," is recognized by agers of both countries. This check occurs close to the nucleus and may represent the adjustment time when the fish changes to a benthic life (Penttila and Dery 1988). A consensus not to count this check as an annulus is presently maintained. However, discrepancies between the Canadian and USA catch-at-age data have been suggested to be the result of counting the settling check as an annulus (Neilson et al. 1991). Neilson et al. (1991) found that age determination biases were not the main cause of these discrepancies, but the evidence was not conclusive.

Lack of agreement in ageing due to checks has been reported by Bailey et al. (1977) for capelin. They were able to show which interpretations were correct by taking otolith measurements and backcalculating to a particular fish size. This study used measurements of annuli and subsequent backcalculations to provide data for quantitatively characterizing settling checks in 5Ze cod otoliths. The specific questions addressed were:

1. What quantitative characteristics identify a settling check and distinguish it from the first annulus?

2. Can annuli (and other) measurements taken from a sectioned otolith be used to aid in "editing" an ager's results?

3. What amount of variation occurs in these measurements due to the position of the section along the sulcal axis? This information is required for any work that is done using cross-sectional measurements of otolith features.

METHODS

Larvae were collected during a June 1985 juvenile gadids survey. Larvae were fixed in 4% formaldehyde and subsequently preserved in 95% alcohol. Larval lengths were measured with calipers to the nearest millimetre. Thirty-four larval otoliths were baked (350°F for 3-7 min) to enhance the features of the sulcus. Measurements were taken on a line at the collum, perpendicular to the anterior/posterior (sulcal) axis (Fig. 1).

For routine age determination, the otoliths from 5Ze cod are presently baked to enhance annuli, then prepared by embedding in resin and sectioning according to the established otolith preparation methods (Strong et al. 1985).

Otoliths used in the calculation of the otolith width/fish length equation were collected during a 1991 spring Georges Bank (GB) groundfish survey. Two fish from each centimetre interval were selected for measurement, for a total of 186 otoliths.

Serially sectioned otoliths were from 1991 GB commercial samples. One hundred of these were sectioned perpendicular to the sulcal axis of the otolith and were used to determine the most accurate mounting and cutting method. Measurements of settling check and first annulus diameters, and total cross-sectional dorsal-ventral width were taken from 22 of these samples (Fig. 1). Serial sections were taken at the standard (M. Strong, DFO, Biological Station, St. Andrews, N.B., pers. commun.) 1.92-mm increment provided by the width of the saw blade, and three of these sections were measured for each otolith. Measurement of three sections assumes that a cut close to the center is usually obtained during the preparation of samples.

Otolith measurements were made under an Olympus SZH microscope and with the use of the Bioscan Optimas 2.0 software package. The software and hardware required will be described by Neilson in a future publication and is referred to as the Image Analysis System (IAS). (Neilson, DFO, Biological Station, St. Andrews, NB; pers. commun.)

Larval otolith measurements were examined at 40x in order to properly identify the collum (Fig. 1). The collum is a readily distinguished external feature which coincides with the location of the nucleus of the otolith (Penttila and Dery 1988). Measurements of annuli and checks were done with a magnification of 25x. Total width was done with 10x. Each of these magnifications required a separate calibration procedure.

RESULTS AND DISCUSSION

BACKCALCULATION

Table 1 lists the otolith widths for 34 larvae between 2.9 and 5.7 cm. The average otolith width was 0.78 mm (± 0.11). Shrinkage due to preservation has not yet been taken into consideration, but is estimated to be 2-5% (Hay 1982; Glenn and Mathias 1987).

Rijnsdorp et al. (1990) reported the relationship between otolith and body size to be slightly curvilinear and used log transformed values to obtain a representative regression line. For backcalculation purposes here, larval measurements were appended to cross-sectional otolith widths of fish between the ages of 1 and 6 (Table 2). The log transformed relationship between fish length and width of otolith is

$$\text{Fish length} = 5.24387(\text{otolith width}^{1.19589})$$
$$n = 119,\ R^2 = 99.24$$

and this is shown in Fig. 2. Outliers in all cases have not been removed as they may represent ageing errors and, if so, information yielded by this study can help in locating otoliths which should be reaged for verification.

SETTLING CHECK AND FIRST ANNULUS

Table 3 lists the measurements of the author's interpretation of "settling checks" which resulted in an average check size of 1.03 mm (±0.22). Figure 2 shows the predicted fish lengths for given widths of otoliths of fish up to 58 cm. The regression equation predicts a fish length at settling to be 5.4 cm. This size agrees with that reported by Lough et al. (1989), where they found 4-6 cm to be the size at which Georges Bank cod began to settle. The average width of the first annulus was found to be 2.59 mm (± 0.45). An annulus this size corresponds to a 16.3-cm fish in the spring of the year. The average 1-yr-old fish in the 1991 spring survey was 19.8 cm.

Figure 3 demonstrates that settling checks and first annuli can quantitatively be distinguished from each other. Each vertical line represents a single otolith in which the settling check, first annulus, and an occasional unidentified or questionable (ann -? wdt) 'check' were observed. The sizes of settling checks and first annuli have little overlap. The questionable 'check' is observed as overlapping with either of the two features. In some cases, identification using its measured size was made later as one of the other two features.

Editing of annuli assignments can be done by using these measurements. For example, in Table 3, features of otolith #1 show correct assignments according to average widths determined. Features of otolith #9 were initially coded as settling check ("sc") and a first annulus ("ann-1"). Once measured, the "ann-1" was found to be closer to the size of the second annulus ("ann-2") shown in Table 4. Otolith #15 shows the correct assignment of the first annulus, although both a questionable annulus (ann-?) and a settling check were detected. Otolith #25 was initially underaged because the first annulus was assigned as "ann-?". After measurements were made, this feature was reassigned as "ann-1," and the subsequent annulus as "ann-2."

MEASUREMENTS OF SERIAL SECTIONS OF OTOLITHS

All data obtained in this study on the width features of otoliths must be viewed in the context of variations caused by the exact cutting position along the sulcal axis. M. Strong (ibid.) found that in a 4-yr-old pollock, total otolith width changed by 2.88 mm during the progression of four standard sections along cutting axis, at which point the first annulus was no longer obvious. He also concluded that the target area along the anterior to posterior axis of a Scotian Shelf cod otolith through which a section will intersect the first annulus to be 5-8 mm.

Results of measurements of serial cross-sections of individual otoliths are shown in Table 4. Within three standard sections, the total otolith width can change by 2 mm ($\bar{X}$ = 1 mm ± 0.99), settling check width varies from an average 0.91-0.96 mm, and first annuli varies between an average of 2.10-2.59 mm. The second annulus was measured to have an average ranging between 4.76-4.99 mm. The increment between the first and second annuli varied between 1.32 and 1.48 mm. This measurement can be used for later growth studies. The size difference between the settling check and the first annulus remains constant throughout the three sections, with an average of 1.35 ± 0.26 mm. More significantly, the number of observations for settling checks decreases from 10 to 4, and 22 to 19 for the first annulus. This effect of cutting sections off-center and "missing" important features such as settling checks and first annuli has been previously discussed by M. Strong (ibid.), and can have a direct effect on age assignment.

GENERAL OBSERVATIONS

Three recurring features were noted for future study:

1) Additional checks were observed between the settling check and first annulus, or directly at the inner edge of the first annulus. These were recorded as questionable (ann-?) checks, with an average size of 1.51 ± 0.39. The expected fish length for this size of otolith would be around 7 cm and would agree with the reported fish length at which demersal juvenile cod change prey items (Mahon and Neilson 1987). Upon re-examination, several of these measured marks could be assigned as a settling check.

2) A "double" or "split" second annulus which may mark onset of maturation or first spawning. O'Brien (1990) states that 50% maturity of Georges Bank cod occurs at age 2 and, if this feature consistently represents the first spawning check, then it could be used to identify first spawning or as an index of size/age at first maturity. It could also be used as a trademark of a second annulus for this stock.

3) Two distinct types of otolith growth patterns. These may represent samples from different areas within Georges Bank, demonstrating different growing conditions, or they may represent fish from adjacent areas. An approach similar to that taken by Ratz (1990) could be used in studying migration by identifying these patterns as biological tags representing different hydrographic conditions.

CONCLUSIONS

The study has successfully quantified settling check and first and second annuli sizes in 5Ze cod and could be used for objective assignment of these features in the future. Care must still be taken that any variations due to sectioning are measured. If substantial variation is observed, this value must be incorporated into the standard deviation of the mean of each measurement taken. Using the average values of each otolith feature shown in Table 4, and applying the standard deviations to the range of values throughout the three serial sections, it can be calculated that:

- settling checks range between 0.51 - 1.31 mm,
- first annuli range between 1.40 - 3.28 mm,
- and second annuli range between 4.34 - 5.38 mm

Therefore it can be concluded that little overlap occurs between the sizes of these features, and that there is a very low probability of their missclassification when using size as a criterium. Also, if the apparent first annulus closest to the center is greater than each of the above ranges, then the age reader can conclude that the previous feature is absent or was missed altogether during sectioning. In the case of missing the first annulus, the age reader should take this into account when assigning the otolith with an age (M. strong, ibid.). The effect of variations in annulus size due to sectioning is expected to increase for annuli of older fish, due to the decrease in interannular distance. Future work is expected to quantify sizes for all subsequent annuli, and similar editing of ages can be done using the equation for the relationship between fish length and otolith width. Similar measurements should be done for each fish species/stock aged as a basis for editing questionable ages or as a learning tool for new agers.

The three additional features noticed during this study (checks, second double rings, annuli patterns) should be investigated further. The double ring formation on the second annulus has already been found useful as a fast way of assessing the absence of the first annulus. When variation due to sectioning is found to be low, interannular distances can provide information on growth rates as related to different areas. All these features show promise in yielding information valuable to ageing, developmental stages, maturity estimates and stock identification.

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REFERENCES

Bailey, R. F., K. W. Able, and W. C. Leggett. 1977. Evidence for the presence of a metamorphic check in capelin (*Mallotus villosus*) otoliths and implications for age determination. J. Fish. Res. Board Can. 34: 2008-2014.

Glenn, C. L., and J. A. Mathias. 1987. Body shrinkage in young walleye, *Stizostedion vitreum*, preserved with AFA, formalin, ethanol and quick freezing. Can. Field-Nat. 101: 408-414.

Hay, D. E. 1982. Fixation shrinkage of herring larvae: effects of salinity, formalin concentration and other factors. Can. J. Fish. Aquat. Sci. 39: 1138-1143.

Lough, R. G., P. C. Valentine, D. C. Potter, P. J. Auditore, G. R. Bolz, J. D. Neilson, and R. I. Perry. 1989. Ecology and distribution of juvenile cod and haddock in relation to sediment type and bottom currents on eastern Georges Bank. Mar. Ecol. Prog. Ser. 56: 1-12.

Mahon, R., and J. D. Neilson. 1987. Diet changes in Scotian Shelf haddock during pelagic and demersal phases of the first year of life. Mar. Ecol. Prog. Ser. 37: 123-130.

Neilson, J. D., M.-I. Buzeta, and J. J. Hunt. 1991. Comparison of catch-at-age matrices employed by Canada and the United States in assessments of stock status of Atlantic cod in 5Z. Can. Atl. Fish. Sci. Advis. Comm. Res. Doc. 91/51.

O'Brien, L. 1990. Effects of fluctuations in stock abundance upon life history parameters of Atlantic cod, *Gadus morhua*, for the 1970-1987 year-classes from Georges Bank and the Gulf of Maine. M.Sc. Thesis, Univ. of Washington.

Penttila, J., and L. Dery. 1988. Age determination. Methods for northwest Atlantic species. NOAA Tech. Rep. NMFS72.

Ratz, H. J. 1990. The assessment of the migration of the Atlantic cod (*Gadus morhua*) between the stocks off east and west Greenland by means of otolith typing. NAFO SCR Doc. 90/34.

Rijnsdorp, A. D., P. I. van Leevwen, and T. A. M. Visser. 1990. On the validity and precision of backcalculation of growth from otoliths of the plaice, Pleunorectes platessa L. Fish. Res. Bd. 9: 97-117.

Strong, M., J. J. Hunt, and R. Robicheau. 1985. A new method for preparing gadoid otoliths. Can. Atl. Fish. Sci. Advis. Comm. Res. Doc. 87/90: 13 p.

Table 1. Cross-sectional (XS) measurement of larval otoliths.

Otolith no.	Larval length (cm)	Otolith XS width[1] (mm)
c90-01	3.4	0.63
c90-02	3.9	0.76
c90-03	4.8	0.90
c90-04	3.8	0.77
c90-05	4.2	0.77
c90-06	3.9	0.80
c90-07	5.7	0.97
c90-08	4.0	0.70
c90-09	4.3	0.93
c90-10	5.5	1.08
c90-11	4.3	0.91
c90-12	3.7	0.76
c90-13	3.8	0.81
c90-14	4.8	0.95
c90-15	4.0	0.78
c90-16	4.2	0.82
c90-18	3.9	0.87
c90-19	4.1	0.82
c90-20	3.4	0.81
c90-21	3.5	0.72
c90-22	4.0	0.77
c90-23	3.6	0.77
c90-24	3.6	0.69
c90-25	2.9	0.52
c90-26	3.8	0.72
c90-27	3.4	0.69
c90-28	4.3	0.81
c90-29	3.6	0.67
c90-30	3.8	0.77
c90-31	3.6	0.76
c90-32	3.7	0.68
c90-33	3.8	0.84
c90-34	3.2	0.62
c90-35	3.1	0.66
Average	3.93	0.78

[1]Total otolith width measured at a line through the collum and perpendicular to the sulcal axis (Fig. 1).

Table 2. Cross-sectional (XS) measurement of fish otoliths.

Otolith no.	Fish length (cm)	Age	Otolith XS width[1]
473	12	1	2.30
480	12	1	2.35
312	13	1	2.41
89	14	1	2.40
90	14	1	2.51
73	15	1	2.70
647	15	1	2.50
84	16	1	2.88
776	16	1	2.71
130	17	1	3.08
1153	17	1	3.02
479	18	1	2.93
1159	18	1	3.16
103	19	1	2.90
128	19	1	3.03
92	20	1	3.27
775	20	1	3.38
95	21	1	3.38
882	21	1	3.08
771	22	1	3.18
781	22	1	3.39
101	23	1	3.79
1193	23	1	3.56
841	24	1	3.32
844	24	1	3.16
115	25	1	4.19
1165	25	1	3.60
822	26	1	2.65
860	26	1	3.33
858	27	1	4.13
1164	27	1	3.89
817	28	1	4.04
1163	28	1	4.21
150	30	2	4.25
985	30	1	4.03
102	33	2	4.66
1064	33	2	5.02
110	34	2	4.98
503	34	2	4.93
149	35	2	5.25
477	35	2	4.69
142	36	2	5.38
1024	36	2	4.63
22	37	2	4.92
86	37	2	4.75
83	38	2	5.22
139	38	2	5.02
72	39	2	5.41
31	40	2	5.17
85	40	2	5.31
19	41	2	5.53
47	41	2	5.31
143	42	2	5.98
265	42	2	5.45
141	43	2	5.79
931	43	2	5.98
435	44	2	5.47
500	44	2	5.99
48	45	2	5.67
87	45	2	6.10
452	46	2	5.78
952	46	2	6.22
39	47	2	5.89
1059	47	4	6.72
80	48	2	6.34
466	48	2	6.21
46	49	3	7.07
548	49	2	6.06
119	50	3	6.64
1127	50	2	6.20
33	51	2	6.66
1122	51	2	6.30
194	52	3	6.52
445	52	3	6.46
431	53	2	6.60
506	53	3	6.64
133	54	3	6.85
158	54	4	7.01
6	55	4	6.98
759	55	3	7.12
352	56	3	7.16
998	56	5	7.17
241	57	4	7.27
1050	57	3	6.79
97	58	6	7.11
670	58	6	6.74

[1]Maximum width of otolith measured from a cross-section at the collum and cut perpendicular to the sulcal axis (Fig. 1).

Table 3. Otolith cross-sectional (XS) widths from commercial samples.

Otolith no.	Fish length (cm)	"sc" width[1] (mm)	ann-? width[2] (mm)	ann-1 width[3] (mm)
1	70	1.06		2.42
2	71	0.61		2.04
3	77		1.33	2.32
4	81			3.25
5	60	1.04		3.31
6	64		1.31	2.76
7	53	0.86		3.23
8	52		1.26	2.07
9	54	0.94		3.82
10	65	0.92		2.16
11	50			2.62
12	50		1.44	2.13
13	63			2.33
14	73	1.20		2.44
15	70	0.75	1.29	2.20
16	75		1.04	2.14
17	99			2.50
18	93		1.67	2.70
19	103			2.67
20	93		1.90	2.65
21	99		1.63	2.98
22	96		1.17	2.10
23	90			2.34
24	83	1.22		2.71
25	89	1.43	2.64	3.63
26	87	1.07		2.35
27	98	0.89		2.19
28	92			2.21
29	90		1.41	2.88
30	82		1.61	2.86
31	68			2.09
32	82		1.44	2.91
33	88	1.19		2.62
34	80	1.23		2.51
Average		1.03	1.51	2.59
S.D.		0.22	0.39	0.46

[1]Width of settling check.
[2]Width of annulus of undetermined origin.
[3]Width of first annulus.

Table 4. Measurements (in millimetres) of otolith features on three serial cross sections.

		Section 1					Section 2					Section 3				
Otolith no.	Fish length (cm)	sc width	ann-1 width	ann-1/ ann-2	ann-2 width	Total width	sc width	ann-1 width	ann-1/ ann-2	ann-2 width	Total width	sc width	ann-1 width	ann-1/ ann-2	ann-2 width	Total width
622	84		1.32	2.01	4.95	8.41	0.89	5.15	1.96	4.90	8.45		1.26	1.95	4.70	7.55
621	98		2.29	1.56	4.72	9.81	0.57	2.43	1.79	4.95	9.34	0.61	2.89	1.74	4.27	9.27
609	103	0.65	2.76	1.22	5.10	10.00		2.47	1.41	5.17	10.92		2.26	1.41	4.97	9.05
610	93	1.20	2.04	1.69	5.70	8.87	1.13	1.96	1.56	5.30	8.80	0.67	1.22	1.67	5.23	8.31
631L	84		2.69	2.06	5.88	9.24		3.04	1.73	5.85	9.01					8.34
631R	84		2.88			9.66		2.77			8.42					8.10
401	64		1.47	1.05	3.81	7.37		2.47	0.89	4.59	7.30		1.94	0.90	4.15	6.59
402	72		1.09	1.66	4.30	8.09		2.72	0.91	5.10	8.85		2.57	1.22	4.80	8.30
403	94		1.98			9.40	0.84	1.88	1.29	4.85	9.65		1.62	1.48	4.79	9.60
407	85		1.17			8.57		2.76	1.43	5.61	9.13		2.10	1.56	5.43	8.80
410	54		1.05	1.91		6.44	0.74	2.75	1.87		6.84					6.46
411	68			1.01	4.80	7.99	1.03	2.73	0.95	4.95	8.20	0.88	2.66			8.18
414	56			1.25	5.14	6.00		2.67	1.16	5.23	6.40		2.62			6.45
416	83			0.98	4.61	8.02		2.59	1.04	4.89	8.73		2.57			8.84
432	109	1.06	2.49	1.67	5.23	11.26		2.50			9.85	1.48	2.79			10.19
434	50	0.94	2.26	1.38	4.89	6.42					5.43			1.85	4.65	6.06
435	57		2.91			7.23	0.73	1.46			5.71		2.39			6.81
450	118		2.34	0.98	4.56	10.61		1.91	1.11	4.32	10.22		1.90			9.55
452	103		2.37	0.98	5.02	9.67	1.22	2.29	1.18	4.97	9.83		1.35	1.40	4.22	8.97
453	42		2.96	1.14	5.01	5.74	0.85	2.63	1.18	5.08	5.31		1.35			4.90
885	71		2.18	0.96	3.87	8.68	1.44	3.17			7.94		1.88			7.01
886	82		2.55			8.51		2.41	1.25	4.36	9.11		3.03	1.11	5.13	7.19
894	94		2.65	1.12	5.22	9.45		2.32	1.11	4.70	8.88		1.51			8.33
Average		0.96	2.17	1.37	4.87		0.94	2.59	1.32	4.99		0.91	2.10	1.48	4.76	
SD		0.23	0.63	0.38	0.55		0.26	0.69	0.34	0.39		0.40	0.69	0.32	0.42	

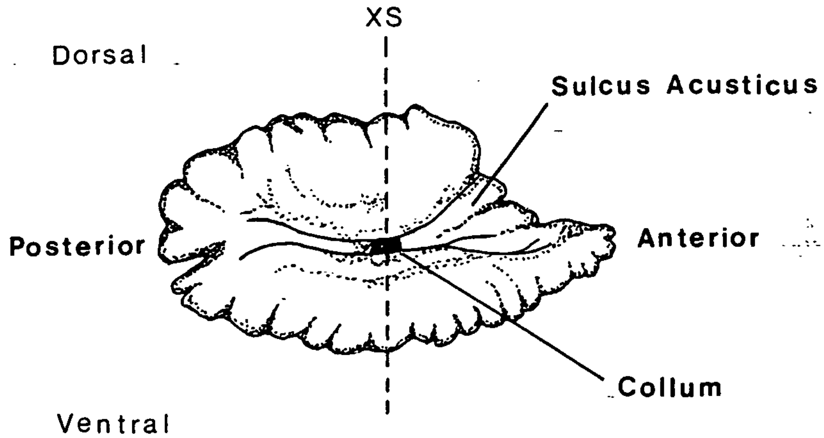

Fig. 1. Sketch of otolith (NOAA Tech. Rep. NMFS72); area of cross-section (XS) is shown.

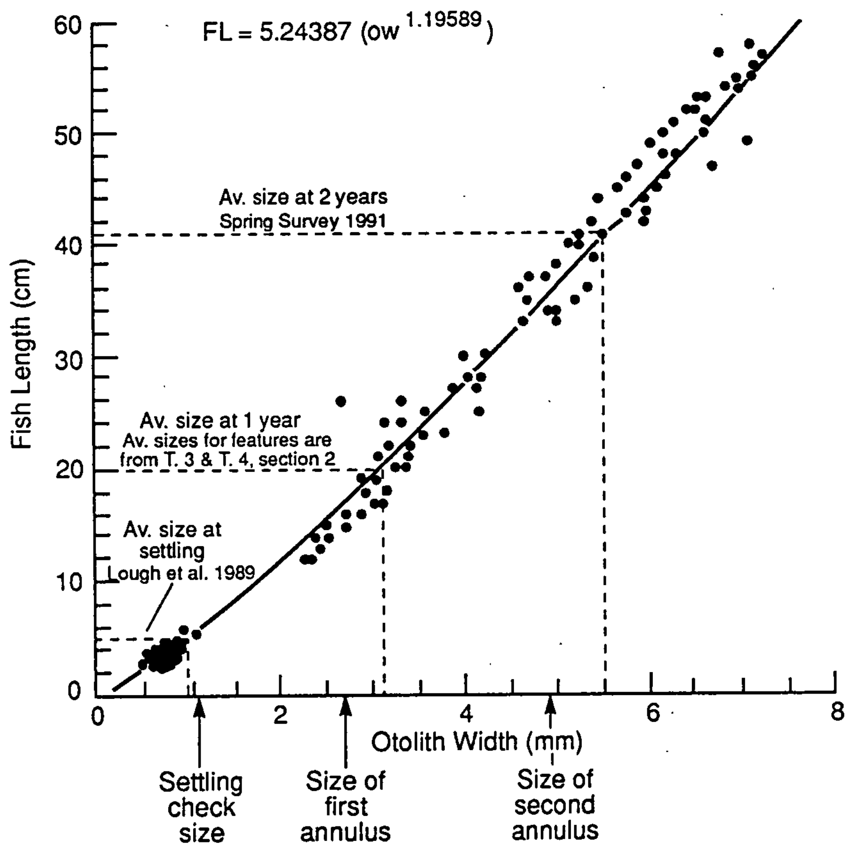

Fig. 2. Regression of fish length on otolith width.

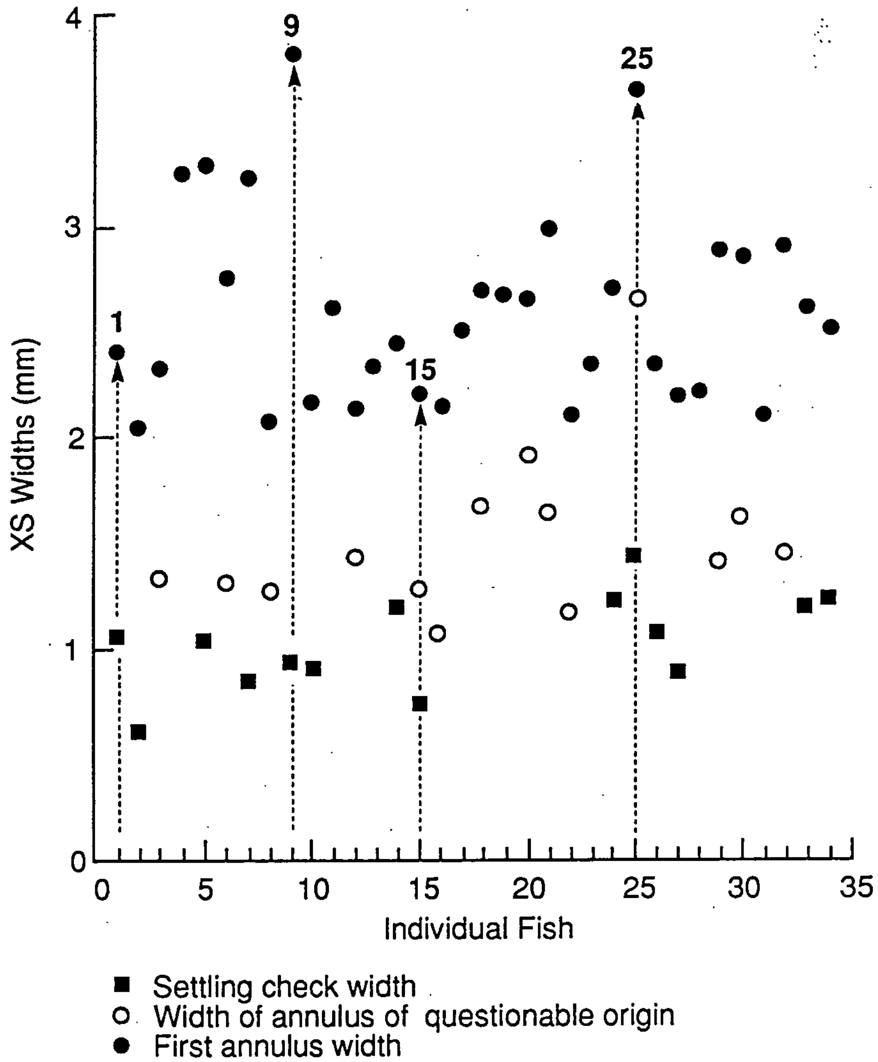

Fig. 3. Cross-sectional (XS) widths of otolith characteristics for 34 individual fish.