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Validation of roughhead grenadier (*Macrourus berglax*) otolith reading.

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ABSTRACT

Roughhead grenadier is becoming an important commercial fish in NAFO Regulatory Area (Northwest Atlantic) and reliable age information is needed for its assessment. *Macrourus berglax* has been aged by otolith reading. Three validation methods were applied: back-calculation, length-frequency analysis and modal progression of an exceptionally large year class.

Size at age estimated by otolith reading, length-frequency analysis and modal progression were consistent, but when estimated by back-calculation it was not. The von Bertalanffy growth model derived from otolith analysis is closer to the Multifan length frequency analysis model and gives values of L*inf* close to the maximum lengths found in catches.

INTRODUCTION

The roughhead grenadier, *Macrourus berglax*, is a deep water fish taken in the by-catch of the Spanish trawl fishery targeting Greenland halibut in the Flemish Pass area (east Newfoundland Grand Bank). This species makes up 6% of the total catch and 8% when discards are taken into account (mean values from 1991-96). These percentages are much higher in the long-line fisheries, representing between 30 and 50 % of the catch (Eliassen, 1983; Savvatimsky, 1984; Jorgensen, 1995; De Cárdenas *et al.*, 1996). At the beginning of the Spanish Greenland halibut fishery in 1990, roughhead grenadier was usually discarded, but as commercial interest has increased, catches are now included in NAFO fisheries statistics.

Little is known of the age structure and growth of *M. berglax*. Savvatimsky (1984, 1994) and Jorgensen (1996) have carried out studies on this species in the Northwest Atlantic, basing findings on age readings from scales. The age structure of *M. berglax* has been estimated by Casas (1994), Sainza (1995, 1996) and Alpoim (1997) from otolith readings of specimens captured in NAFO Divisions 3L and 3M. Eliassen (1983) also performed age estimation by otolith reading from roughhead caught in the continental slope of Norway.

Age determination of fish is essential in fisheries management and its procedures must be reliable and provide valid results. Validation tests are necessary to every study that involves the extraction of age data from calcified fish structures (Casselman, 1983). With this aim, the present study deals with the validation of the length-age key from otolith reading using three methods: back-calculation, length-frequency analysis and modal progression of an exceptionally large year class.

MATERIAL AND METHODS

The lengths of roughhead grenadier were measured to the lower 0,5 cm from tip of snout to base of first anal-fin ray, anal-fin length (AFL), the standard measurement adopted by NAFO in June 1980 (Atkinson, 1991).

Both sexes have been validated separately since the length at age for males is lower than for females from eight-nine years. Mean lengths at age and by sex were obtained following four methods:

1. Length-age key by otolith reading

A mean length-age key was made using otoliths from two bottom trawl surveys, one commercial bottom trawl and one long-line survey. In this way it has been possible to cover the whole length range, as catches from these latter two include specimens of greater length than those obtained in the former (Table1).

The otolith section reading procedure described by Casas (1994) was followed. Otoliths were broken through the nucleus and read by transmitted light. A magnification factor (x20) was used to distinguish the rings, increasing this amplification for the edges of otoliths from large specimens.

Intercalibration of readings between three readers was performed, reaching a level of agreement of around 80% between each pair of readers, differences of ± 1 year being found in otoliths of less than 10 years, and of ± 1 or 2 years in those of more than 10 years. This intercalibration includes the AFL-age key of 1996 (Alpoim, 1997).

2. Back-calculation

To obtain mean lengths at age through back-calculation, otoliths from two bottom trawl surveys, one commercial bottom trawl and one long-line have been used, covering a broad range of lengths (Table1 and Table 2).

As the growth of the otolith is not isometric throughout the life of the fish and annual growth rings are not formed similarly on all planes of the otolith, a criteria was adopted of taking measurements of the radii of the rings of the longer or ventral wing of the section of each otolith broken through the nucleus (Figure 1). The ventral wing was chosen because the proportions of the rings remain constant on this side, and because it is on the ventral edge of the sulcus acusticus that reading of rings is best performed, and thus the point from which these rings are best traced to the edge of the otolith.

The measurements were taken from the focus to the outer edge of each hyaline or non-growth ring, with an accuracy of ±0,01 mm. The focus was established as being half the diameter of the first ring. Measurements were taken from the image of the otolith amplified on a television screen taken from a camera incorporated into the binocular microscope.

An examination was made of the edge of the otolith to determine when the hyaline ring had fully formed, but as otoliths from small specimens were not available throughout the year, this could not be established. For this reason it was decided that the measurements of the radii of the outer-most ring of each otolith should not be included.

To infer the relationship between size of the ventral radius of the otolith section and that of the body (AFL), a regression was performed by the least squares method. The statistics F-ratio value (Fisher-exact test) and determination coefficient were provided. The resulting equation was applied to the radius of each ring measured.

3. Length-frequency analysis

The Multifan 32 program (Otter Research Ltd., 1992) was applied to the length distributions in number of the commercial catches pondered to the haul and by month from March 1993 to December 1994.

Having run the program with different values of the von Bertalanffy K parameter and number of age classes, and based on the best fits of theoretical modes to the length distribution and to the most realistic values of *Linf*, the following search was chosen:

Females: Initial systematic search: without mean length at age constraints; K values of 0.02, 0.04 and 0.08, and age classes 15, 16 and 17. Each systematic search is associated with a particular structural hypothesis relating to growth and fishing selectivity. Three models were fitted to the length-frequency data. Model 1 had constant standard deviation at age, model 2 had age-dependent standard deviation in length at age, and model 3 had both age-dependent standard deviation in length at age and sampling bias in first cohort.

Males: Initial systematic search: without mean length at age constraints; K values of 0.05, 0.07, 0.09 and 0.11 and age classes 11, 12, 13 and 14. The same three models were applied as for females.

An adjustment to the attributed individual age was performed for Multifan length at age since length at age estimated by the other three methods was considered to be: determined age + 0.5 (sampling time was the end of the 2nd quarter – beginning of the 3rd quarter).

4. Modal progression of an exceptionally large year class

The mean lengths were taken from a cohort which appears to be well represented in the July bottom trawl surveys from 1992 to 1997 (Sarasua *et al.*,1998b). Mean lengths were obtained for this cohort from 1993 to 1997, pondering total catches to the length-age key from each survey: AFL-age key of 1994, 1995, 1996 and 1997 (Casas, 1995; Sainza, 1996; Alpoim, 1997; Sarasua *et al.*,1998a). For 1993 the key used was the mean of the 1996-1997 surveys. It was not estimated for 1992 because data were not differentiated by sex.

A sample of otoliths from the two bottom trawl surveys of 1994 and 95 were re-read, and a level of agreement with the AFL-age keys of Casas (1995) and Sainza (1996) of around 50% was found, where the new readings were of 1, 2 and 3 years more for ages over 5, but practically the same for ages less than or equal to 5 years. Given that it is the 1990 cohort, we considered it best to apply these keys to their corresponding catches.

Annual growth parameters of the von Bertalanffy growth curve were estimated for each sex from AF length-age keys using the SPSS program (version 61.3). Multifan estimates the von Bertalanffy parameters from length frequency data.

RESULTS

The anal fin length-age key by sex is presented in Table 3. As the aim of this length-age key is not to ponder the catch, but rather to obtain a broad range of ages, otoliths from the commercial bottom trawl and long-line fisheries have allowed the sample of large specimens to be increased, and male ages from 2 to 12 and ages 3 to 16 in females are considered to be adequately covered.

The best fit between the size of the otolith radius and anal fin length was that of a potential curve. The equation obtained for males was:

$$\text{AFL(cm)} = 4.7873 * \text{radius length(mm)}^{1.1855} \quad (N=159,\ r^2=0.60,\ F=237.2,\ P< 0.001)$$

And for females:

$$\text{AFL(cm)} = 3.7738 * \text{radius length(mm)}^{1.5091} \quad (N=257,\ r^2=0.57,\ F=342,\ P< 0.001)$$

In Figure 2 the fitted curves for both sexes are represented. Both show great dispersion of data, and there is an inflexion in the distribution of points at around 14 cm in males and 18 cm in females.

In the length-frequency analysis the best fitting models for both males and females included age-dependent standard deviation in length at age that decreased with age. Sampling bias in first cohort did not produce an improvement in the fit. Model 2 discriminated 14 and 17 age-classes for males and females respectively.

The mean AFL at age and standard deviation obtained by the four methods is presented in Table 4. Mean length at age is practically identical for both sexes up to 8-9 years, and it is from this age that a greater mean length is observed in females. The mean AFL obtained through the length-age key, length frequency analyses and modal progression for both sexes are very similar. This is not so in the case of the mean AFL obtained through back-calculation as is best appreciated in Figure 3.

The differences between back-calculation and the other three methods appear in females from 1 to 1.5 years for ages less than 10 and of 2 years for ages over 10. In males these differences are greater, above all from 5 years, in which differences of up to 5 years appear.

Annual growth parameters of the von Bertalanffy growth curve estimated for each sex from AF length-age keys and from length frequency data by the Multifan program appear in Table 5. The parameters estimated from the mean lengths of the AFL-age key were obtained in two ways, one with restrictions and one without. For the former assumption *Linf* was taken to be the AFL maximums found in the length frequency data and To = 0, since the Multifan program estimates K and *Linf.* with To = 0.

DISCUSSION

Size at age estimated by otolith reading, length-frequency analysis and modal progression were consistent, but when estimated by back-calculation it was not.

The relationship between size of the structure and anal-fin length, although significant, is very low, fundamentally due to not having measured the maximum length of the otolith, but rather the ventral radius of the transverse section of the otolith at its nucleus. This section is obtained on cutting the otolith, but the cut is not always exactly at the focus and neither is it always exactly transverse. This, together with the numerous lobes on the ventral edge of the otolith, brings about great variability in the size of the ventral radius of the otolith section.

This relationship is clearly allometric and so the power function fitted the data better than did any other function. This relative growth is linked to differences in growth on reaching sexual maturity, and in fact the inflexion coming about in the distribution of the cloud of points of both sexes is due to this factor. In the case of females this inflexion comes at around 18 cm, the length at which sexual maturity begins to occur, L_{50} being reached at 26.2 cm (Murua and Motos, 1997). The dispersion which occurs in this length range would be due to differential growth of specimens until all of these reached sexual maturity.

The bad fits obtained lead us to consider revising the methodology used in back-calculation, following the review of back-calculation of Francis (1990).

Comparing our mean AFL by otolith reading with those obtained by Savvatimsky (1994), in which total length was converted to AFL using Atkinson's formula (1991) and those of Jorgensen (1996) by scale reading, mean lengths at age for males practically coincide with those of the former, slightly higher than the latter. With Jorgensen (1996) the differences are of less than 0.5 years, and the greatest differences are given in ages from 4 to 6, but we think this is due to the scarce number of specimens used by this author to estimate the mean lengths of these ages. In females our mean sizes are higher than those obtained by both authors, the differences being of less than 1 year up to age 13, and higher beyond this age.

To age 8-9 the size at age is the same for males and females, and thereafter the size for males is lower than for females. Savvatimsky (1994) and Jorgensen (1996) found that the differences between sexes in size at age come about from 10 years onwards.

These differences in mean size at age and in the age at which males begin to be smaller than females, found between our work and those of the authors previously mentioned, are surely due to the latitude of the sampling areas where the specimens were obtained. The grenadiers from Jorgensen's study come from NAFO Divisions 1ABCD and those of Savvatimsky are from NAFO Divisions 0, 2GHJ and 3K where The Labrador Current, carrying cold, low-salinity water has a very strong influence, while our specimens come from Flemish Cap and Flemish Pass, where the waters are influenced by The North Atlantic Current, which brings northward flowing, warm, high-salinity water. The origin of these slope waters would be in the interaction between the Gulf Stream rings and Newfoundland shelf waters (Drinkwater 1996). This temperature difference would cause slower growth, but to a greater size and a delay in reaching sexual maturity, since the length of females corresponding to 9 years in our specimens is precisely the length at which sexual maturity has begun to come about (Murua and Motos, 1997).

If a species grows according to the von Bertalanffy growth model and if a broad range of ages is available, then the length at age data may be applied to the model to ascertain whether they produce realistic growth parameters, e.g., mean asymptotic length, *Linf* (Casselman, 1989). The growth model derived from otolith analysis is closer to the Multifan model (Table 5) and gives values of *Linf* close to the maximum lengths found in catches. The von Bertalanffy parameters estimated for both sexes by Sainza (1995) are very similar to ours and to those found by Savvatimsky (1994) for males, but not for females, in which *Linf* is exaggeratedly high and the value of K consequently very low.

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Table 1.- Number of otoliths by sex used to construct the AFL-age key and in back-calculation.

Sample source	Year	Month	NAFO Div.	AFL-age key		Back-calculation	
				Males	Females	Males	Females
Bott. Trawl survey	1995	July	3M			37	67
Long-line survey	1995	April-May	3LM	20	49	16	42
Bott. Trawl survey	1996	July	3M	221	169	-	-
Commer. bott. trawl	1996	March-April	3LM	11	77	11	17
Bott. Trawl survey	1997	July	3M	159	231	100	141
Total				411	526	164	267

Table 2.- Number of otoliths by size group (cm) and by sex used in back-calculation.

AFL size group (cm)	Males	Females
4-6	3	4
7-9	11	18
10-12	21	44
13-15	39	42
16-18	36	29
19-21	39	37
22-24	14	32
25-27	1	25
28-30		19
31-33		11
34-36		5
37-39		1
Total	164	267

Table 3.- Mean anal-fin length-age key by sex of roughhead grenadier.

Males

AFL	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
	Age class														
3	1														1
4	4														4
5	1														1
6	3	3													6
7		4													4
8		6	3												9
9		2	4												6
10			11	2											13
11			2	1	1										4
12				11	13	1									25
13			1	11	17	5									34
14				4	31	13	1								49
15					10	20	6								36
16					5	13	12	4							34
17						6	8	6	6						26
18							11	11	8	6	1	1			38
19							2	8	13	14	6				43
20								3	7	14	11	2	1		38
21								2	2	6	7	4			21
22								2	2	5	2	1		2	14
23											1	3			4
24													1		1
Total	9	13	21	29	77	58	40	36	38	45	28	11	2	2	411

Females

AFL	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total
	Age class																									
4	2																									2
5	4																									4
6		4																								4
7		16																								16
8	1	10	2																							13
9		2	7	1																						10
10			10	2																						12
11			6	6																						12
12				10	6	1	1																			18
13				10	10	5																				25
14				3	24	16	2																			45
15					10	17	5	1																		33
16					3	20	7	1																		31
17						8	9	3																		20
18						2	5	6	1																	14
19							6	9	7	3	1															26
20								7	8	6	1															22
21								2	3	6	1	1														13
22									5	9	7	2	1													24
23									2	5	4	2														13
24									2	2	6	7	2		1											20
25										1	1	3	3	1	1	1										11
26									1	1	4	2	1	5	1	1										16
27												3	6	2	1		3									15
28												3	6	5	3	1		3								21
29													1	4	7				2							14
30												1		1	3	1	3		1	1						11
31												1			1	3	3		2						1	11
32													1	1	1		1	1	1	1	1					8
33													2		1	1	1	1	1	1				1		9
34														2	1	2	2	1	2	1		2				13
35																	1	2	1	1				1		6
36														1	1					1	1					4
37															1			1								2
38																				1				1		2
39																		2							1	3
40																			1				1			2
41																				1						1
Total	7	32	25	32	53	69	35	29	29	33	25	25	23	22	23	10	14	11	11	8	2	2	1	3	2	526

Table 4.- Summary of results by sex, estimated by the four methods.

	AFL-age key			Back-calculation			Length-frequency analyses Multifan			Modal progression Cohort 1990		
Males	N	Mean	SD	N	Mean	SD	N	Mean	SD	Year	Mean	SD
AGE 1				163	1.9	0.50						
2	9	4.7	1.05	105	3.7	1.00						
3	15	7.5	0.96	91	5.4	1.33	2679	8.4	0.91	1993	7.8	0.73
4	21	9.8	1.11	82	6.8	1.56	5380	10.3	0.84	1994	9.8	0.87
5	29	12.5	1.00	71	8.0	1.66	7608	12.1	0.77	1995	11.9	1.02
6	77	13.7	1.14	55	9.6	1.48	13312	13.7	0.72	1996	13.8	1.17
7	58	15.0	1.17	39	10.8	1.21	17083	15.1	0.68	1997	14.7	1.10
8	40	16.7	1.23	27	11.5	1.32	28213	16.4	0.64			
9	36	18.4	1.55	15	12.1	1.64	31473	17.7	0.61			
10	38	18.9	1.31	11	12.5	1.37	20577	18.7	0.58			
11	45	19.8	1.17	5	13.3	1.39	12168	19.7	0.55			
12	28	20.2	1.08	3	14.3	0.36	5933	20.6	0.53			
13	11	21.2	1.47	1	14.9	-	3366	21.5	0.51			
14	2	22.0	2.00				1718	22.2	0.5			
15	2	22.0	-				602	22.9	0.48			
16							537	23.5	0.47			
Total	411			668			150649					
Females	N	Mean	SD	N	Mean	SD	N	Mean	SD	Year	Mean	SD
AGE 1				267	1.4	0.66						
2	7	5.1	1.25	150	3.1	1.45						
3	32	7.3	0.77	130	4.8	2.11				1993	7.3	0.66
4	25	9.8	0.89	116	6.5	2.65	6594	9.9	1.11	1994	9.8	0.93
5	32	12.1	1.18	80	8.7	3.20	9517	11.9	1.05	1995	12.4	1.36
6	53	13.9	1.02	111	10.2	3.36	16070	13.9	1	1996	14.2	1.14
7	69	15.2	1.28	75	12.6	3.27	25112	15.7	0.95	1997	15.0	1.36
8	35	16.7	1.65	53	14.7	3.18	40092	17.5	0.91			
9	29	18.7	1.39	41	17.1	2.99	45468	19.1	0.67			
10	29	20.8	1.86	28	18.7	2.93	24853	20.8	0.83			
11	33	21.7	1.66	23	19.9	2.94	14071	22.4	0.8			
12	25	23.2	1.80	21	20.7	3.27	17115	23.9	0.76			
13	25	25.3	2.44	17	21.8	2.02	13987	25.3	0.73			
14	23	27.3	2.64	12	22.5	2.02	7980	26.7	0.71			
15	22	28.7	2.87	9	23.0	2.15	4328	28.1	0.68			
16	23	29.7	3.07	8	23.8	2.09	3584	29.4	0.66			
17	10	30.3	2.97	7	24.4	2.22	2453	30.6	0.64			
18	14	30.9	2.53	5	24.8	2.40	1328	31.8	0.62			
19	11	33.5	3.94	5	25.4	2.40	662	33.0	0.6			
20	11	32.5	3.06	1	22.8	-	709	34.1	0.58			
21	8	34.9	3.26									
22	2	34.0	2.00									
23	2	34.0	-									
24	1	40.0	-									
25	3	35.3	2.05									
26	2	35.0	4.00									
Total	526			1159			233923					

Table 5- Estimated parameters of the von Bertalanffy equation from length frequency analysis and mean anal-fin length-age key

	Length-frequency analysis Multifan program		Anal fin length-age key			
			With no restrictions		With restrictions (To = 0)	
	Males	Females	Males	Females	Males	Females
K	0.095	0.039	0.152	0.056	0.076	0.064
Linf	29.5	61.7	24.85	46.99	36	44.5
To	0	0	0.647	-0.129	0	0
Restrictions					*Linf* = 36	*Linf* = 44.5

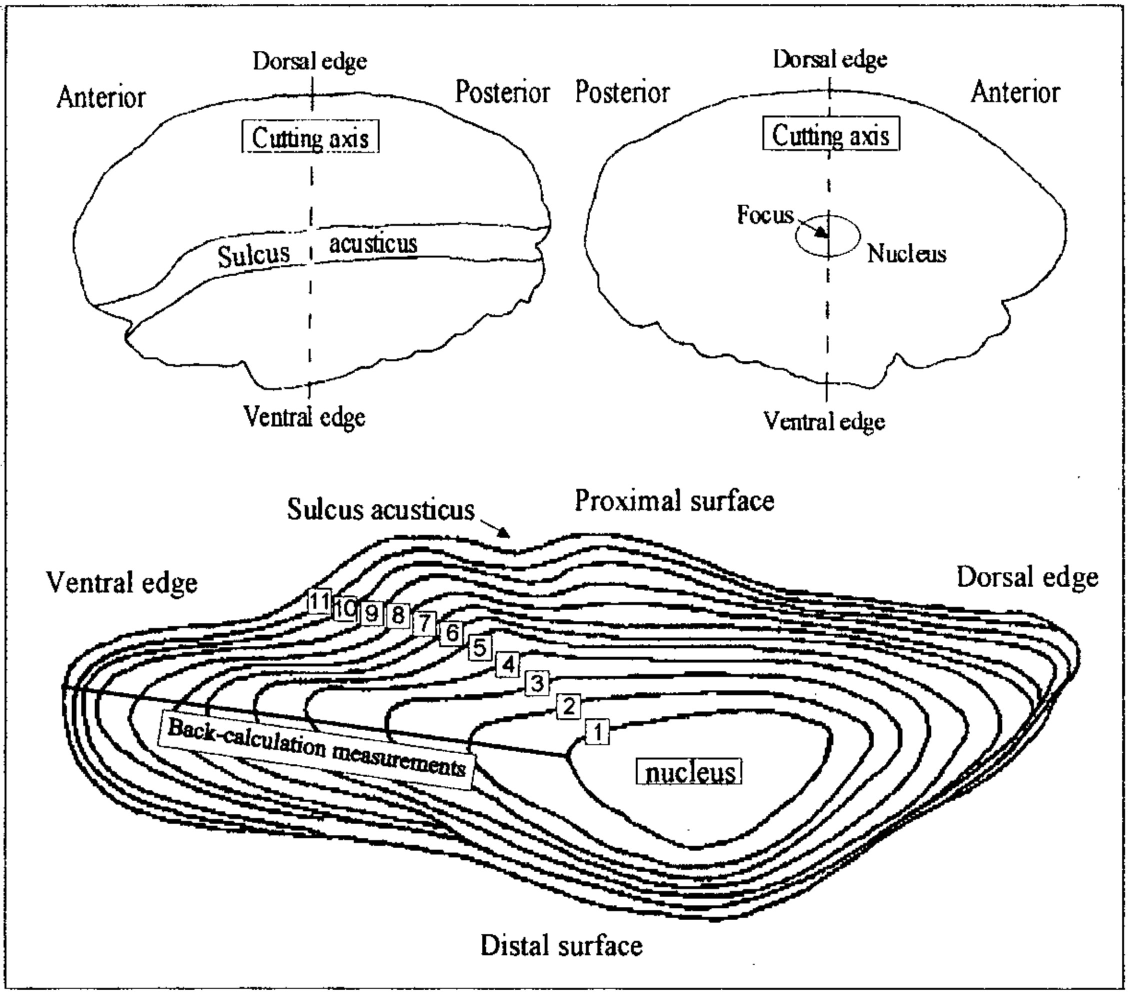


Fig. 1- Views of a right otolith and transverse section from *Macrourus berglax* illustrating orientation and basic structures

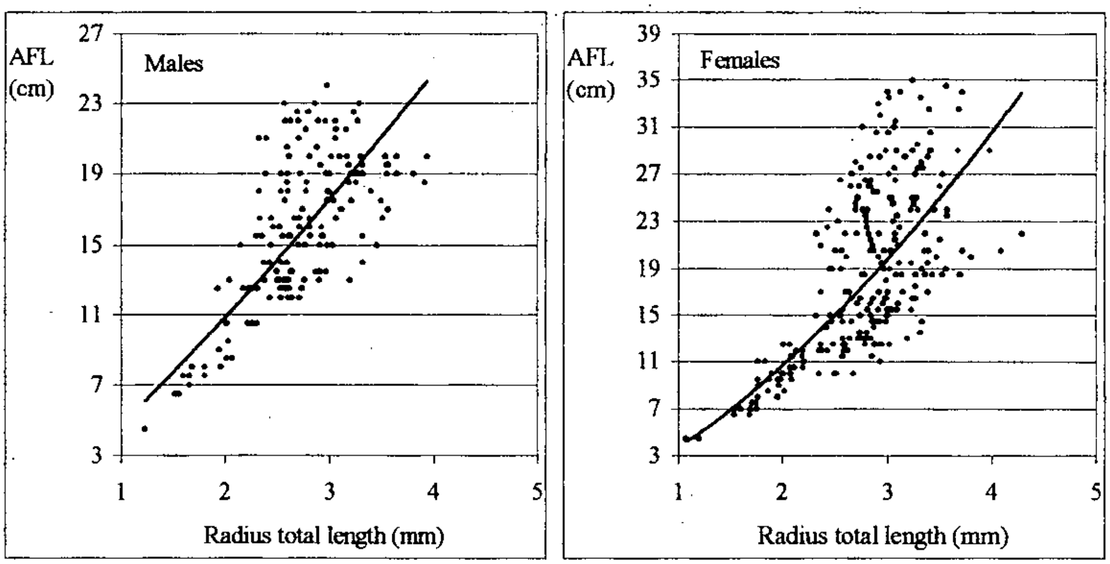
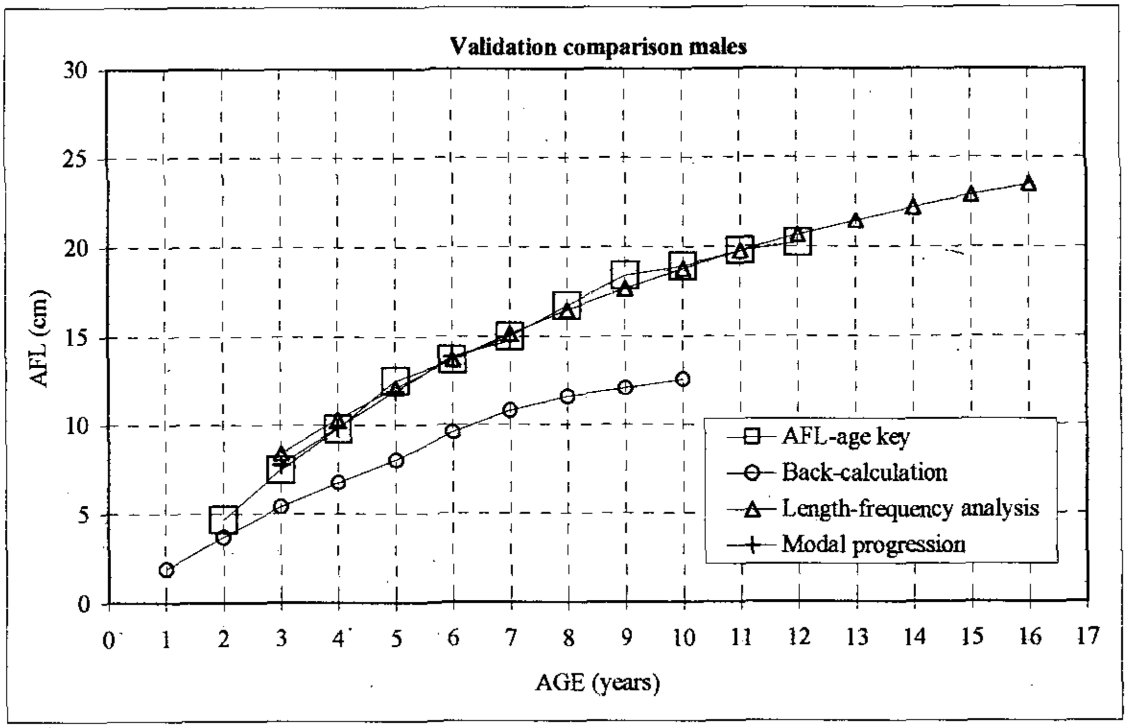


Fig. 2- Relationship between anal-fin length (AFL) (cm) and total ventral radius (mm) by sex used in back-calculation



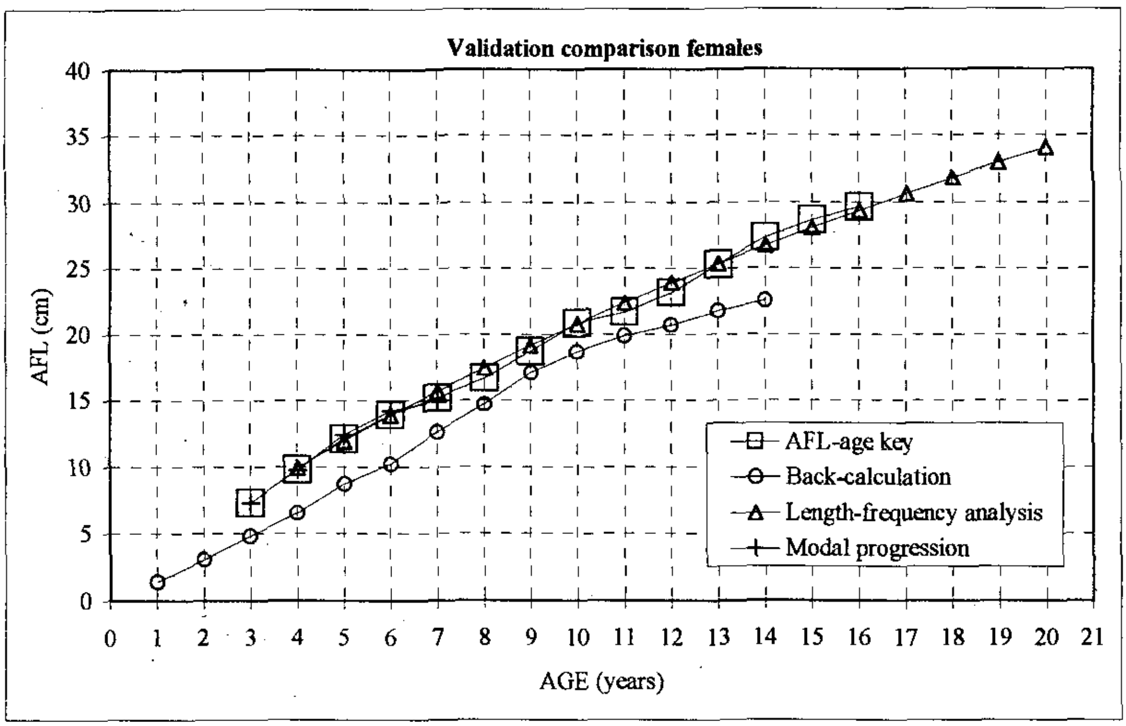


Fig.3- Comparison of mean anal-fin length by age, obtained using the four different methods.