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Inter-laboratory verification of ageing consistency for Atlantic herring (*Clupea harengus harengus*) otoliths

Vérification inter-laboratoire de la concordance dans la détermination d’âge d’otolithes de hareng (*Clupea harengus harengus*)

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ABSTRACT

An inter-laboratory verification of ageing consistency for Atlantic herring (*Clupea harengus harengus*) of the Gulf of St. Lawrence was undertaken by two laboratories, the Maurice Lamontagne Institute (MLI) in Mont-Joli (Quebec) and the Gulf Fisheries Center (GFC) in Moncton, (New Brunswick). The new primary age reader and the secondary age reader of MLI were compared with the highly experienced GFC age reader. The two laboratories aged a total of 200 whole otoliths from the 2010 commercial fishery conducted in the northern Gulf of St. Lawrence; half of the otoliths were from NAFO Division 4R and half from NAFO Division 4S.

Results are presented in terms of percentage agreement, total coefficient of variation (CV), Bowker's test of symmetry, and age bias plots. The primary IML reader compared to the GFC reader resulted in 81.1% agreement with a CV of 2.05%. The secondary IML reader compared to the GFC reader yielded 66.5% agreement with a CV of 3.58%. The comparison of the two IML readers resulted in 71.8% agreement with a CV of 2.74%. In all three comparisons, Bowker's test showed that there was no significant asymmetry. The levels of precision reflect the difficulty in the age determinations of these herring, being from catches in the summer and with a predominance of older fish.

RÉSUMÉ

Dans le but de mesurer la concordance entre deux laboratoires dans la détermination de l'âge du hareng atlantique (*Clupea harengus harengus*) du golfe du St-Laurent, une vérification des lectures a été réalisée par l'Institut Maurice-Lamontagne (IML) de Mont-Joli (Québec) et la Centre des pêches du Golfe (CPG) de Moncton (Nouveau-Brunswick). Le nouveau premier lecteur d'âge et le deuxième lecteur de l'IML ont été comparés au lecteur de longue expérience du CPG. Les deux laboratoires ont fait la lecture d'un total de 200 otolithes provenant de la pêche commerciale pratiquée dans le nord du golfe du Saint-Laurent en 2010; la moitié de ces otolithes provenait de la Division 4R de l'OPANO et l'autre moitié de la Division 4S.

Les résultats sont présentés en termes de pourcentage de concordance, de coefficient total de variation (CV), du test de symétrie de Bowker et de graphiques du biais des âges. La comparaison entre le premier lecteur de l'IML et celui du CPG indique un niveau de concordance de 81,1 % avec un CV de 2,05 %. La comparaison du deuxième lecteur de l'IML et celui du CPG montre un niveau de concordance de 66,5 % avec un CV de 3,58 %. Enfin, la comparaison des deux lecteurs de l'IML montre un niveau de concordance de 71,8 % avec un CV de 2,74 %. Dans les trois comparaisons, le test de Bowker a montré qu'il n'y avait pas d'asymétrie significative. Les niveaux de précision reflètent la difficulté dans la détermination d'âge de ces harengs provenant des prises de l'été avec une prédominance de vieux poissons.

INTRODUCTION

The Atlantic herring (*Clupea harengus harengus*) stocks in the Gulf of St. Lawrence are managed by two DFO regions: Quebec for NAFO Divisions 4R and 4S, and Gulf for NAFO Division 4T. Ageing of otoliths is carried out by both regions in order to determine the demographic structures of the stocks, length at age and catch at age in commercial and research samples for stock assessments. In the northwest Atlantic, herring stocks are either spring-spawners or fall-spawners and during sample analyses, the spawning season is assigned according to gonad maturity and characteristics in the otoliths distinct to each group (McQuin 1989).

The Quebec herring stock assessments for 2005 to 2009 were done without age data due to the absence of a primary age reader. In 2008, a new primary age reader at Maurice Lamontagne Institute (MLI) underwent training with the highly experienced primary reader at the Gulf Region's Gulf Fisheries Centre (GFC), using samples from both regions. MLI also adopted GFC's method of embedding the otoliths in resin and reading otoliths under water immersion. A reference collection of 700 otoliths from Quebec samples was created for the use of the MLI readers. The primary MLI reader has since then carried out the ageing of Quebec's 2005 to 2010 samples, followed by the secondary reader.

Since MLI and GFC are involved in the ageing of adjacent herring stocks, consistency in the interpretation of ages from otoliths must be ensured during ageing. Inaccurate age determinations will have an impact on estimations of population dynamics and stock assessments. The monitoring of ageing consistency and checking for biased errors insures that the age interpretation method does not drift over time.(Campana 2001).

Quality control efforts have been a carried out regularly since IML's new primary herring otolith reader was trained in the fall of 2008. Along with the use of the reference collection and the exchange of samples, high definition cameras and imaging software are used to exchange photos of otoliths between the IML and GFC. Precision tests are conducted regularly between the primary and secondary readers of IML to check for errors and bias and corrections are made to the age data.

A way to verify the level of inter-laboratory precision is by readers from each laboratory ageing the same set of fish independently. Precision levels can then be calculated from the age readings for each fish. The results are provided to scientists who prepare stock assessments, so that they may consider this potential source of variability.

The purpose of this report is to provide the results of the 2010 inter-laboratory verification of ageing consistency for Atlantic herring of the Gulf of St. Lawrence. These results apply to recent ageing preformed at MLI and for samples aged in preparation for the 4S herring stock assessment meeting in February 2011.

METHODS

At MLI, the primary age reader conducted the ageing of herring, following standard ageing methods and criteria (Penttila and Dery 1988) and specific information received during the training at GFC. The herring used for ageing were selected randomly (50) from each commercial landing that was port sampled. Prior to ageing, the preparation involved placing the pairs of whole otoliths in 50-hole trays and embedding them in clear resin (Cytoseal ™). During ageing, all fish were assigned a January 1st birth-date and the spawning season was determined for each otolith.

After the ageing at MLI of otoliths from 2010 herring samples of Quebec commercial catches, 200 otoliths were selected to be sent to GFC for the ageing comparison, 100 from each of the NAFO Divisions 4R and 4S. Herring in the 4R samples were caught by purse seine on May 26th near Curling Nlfd (n=50) and by gillnet on July 15th near Sandy Cove Nfld (n= 50). The 4S herring were caught in herring traps on July 19th, near Brador QC (n= 50) and on July 25th near Old Fort Bay QC (n= 50). MLI's secondary reader, who has been reading herring otoliths since 1999, also participated in the comparison. Trays of embedded whole otoliths were passed between the readers, and the age was not exchanged until after the third reader had completed age determinations for all fish. During ageing, all age readers had access to data regarding fish length, collection date, and location.

The ageing data used in precision calculations was annuli counts. For spring-spawner herring, the annuli count corresponds to the age, since the first annulus is visible (Figure 1A). But for fall-spawner herring, since the first annulus is not visible (Figure 1B), the convention is to add 1 to the annuli count to obtain the actual age for the analysis data in stock assessments. Although Atlantic herring can reach about 15 years old (a few up to 18), at MLI herring otoliths are aged up to an annuli count of 11, after which the count is recorded as 11+.

Results for each exercise are presented in terms of percent agreement, coefficient of variation (CV), age-bias plots, and age-frequency tables (Campana et al. 1995, Campana 2001, FBP 2008). An additional test, Bowker's test of symmetry (Hoenig et al. 1995, FBP 2008), is also presented to show the level of significance of ageing bias when the percent agreement is below 90 %. Age-bias plots show the average age attained by the 1st MLI age reader versus the GFC reader's age, the 2nd MLI reader versus the GFC reader, and the 1st MLI reader versus the 2nd MLI reader. Age-frequency tables compare the numbers of otoliths at each age for the three age readers. If two ages were not assigned to a fish for any reason, the fish was excluded from calculations of precision.

RESULTS

The results of the comparison between MLI's first reader (RM) and GFC's first reader, with 190 herring aged, show an acceptable level of agreement 81.1% with a CV of 1.95% which is well below 5%. The age-bias plot shows the average annuli count attained by the GFC age reader (CM) versus the annuli count attained by the 1st MLI age reader (RM) (Figure 2A). Bowker's test, used to detect departure from symmetry within the age-frequency table, shows that there was no significant asymmetry (Figure 2B). The age-frequency table, which groups the two readers' annuli counts (Figure 2C), shows the highest numbers in the boxes along the main diagonal where both readers were in agreement. The numbers above this diagonal indicate herring that were given higher annuli counts by the GFC age reader and those below the diagonal indicate higher annuli counts given by the MLI age reader. Figure 2D shows the frequency of differences in annuli counts. For 155 herring the difference was 0. Positive differences indicate a higher annuli count given by the GFC reader, while negative differences indicate a higher annuli count given by the MLI age reader.

The results of the comparison between MLI's two readers (RM and JG), with 195 herring aged, show a level of agreement of 71.8% and the CV of 2.74% is well below 5%. The age-bias plot shows the average annuli count attained by the MLI second age reader (JG) versus the annuli count attained by the first MLI age reader (RM) (Figure 3A). Bowker's test of symmetry shows there was no significant asymmetry (Figure 3B). The age-frequency table is shown in Figure 3C and the frequency of differences in annuli counts is shown in Figure 3D. For 140 herring the difference was 0.

The results of the comparison between GFC's reader (CM) and MLI's second reader (JG), with 185 herring aged, show a level of agreement of 66.5% and the CV is 3.58%. The age-bias plot shows the average annuli count attained by the MLI second (JG) age reader versus the annuli count attained by the GFC age reader (CM) (Figure 4A). Bowker's test of symmetry shows there was no significant asymmetry (Figure 4B). The age-frequency table is shown in Figure 4C and the frequency of differences in annuli counts is shown in Figure 4D. For 123 herring the difference was 0.

The length distribution and mean length per annuli count for the three otolith readers is presented in the Appendix 1. Only herring designated as fall-spawners are displayed; spring-spawners represented less than 2% of the otoliths aged.

DISCUSSION

The agreement level of 80% has been considered adequate by laboratories such as the Northeast Fisheries Science Center at Woods Hole (Campana 2001). In this study, the level of precision between the primary readers of the two laboratories was just above this value, while the comparisons involving the secondary MLI reader were below.

Total coefficient of variation, or CV, (Chang 1982) is now considered a more reliable method of measuring agreement, and CVs of below 5% are generally considered acceptable for species such as herring (Campana 2001). In all three comparisons of this study, the CV was below 5%. If the annuli counts had been converted to age in the fall-spawners of our samples (age = annuli count plus one), the resulting CVs would be even lower, since age differences in older fish contribute less to the total CV.

In the three age-bias plots, the points can be seen to fall on or very near the diagonal line, which indicates no systematic bias. The 95% confidence intervals, for most points, are small and bracket the diagonal line. The value at 11 groups counts of 11 and more, therefore bias is reduced.

In the age-frequency tables, few otoliths fall outside the diagonal boxes for annuli counts less than 7 and there are some otoliths on either side of the diagonal at 7 and older. In all three comparisons, Bowker's test of symmetry was non significant. The totals across the bottom and on the right side, indicating the distribution of annuli counts, show a peak at 8. The distributions of length as well as the mean length per annuli count are similar for the three readers, with the peak length of 33 cm corresponding to 9 year-old herring (age is annuli count plus 1 for fall spawners). This reflects the age structure of the landings since the otoliths were from random samples of landings rather than length stratified samples as used for southern Gulf of St. Lawrence herring which gives a fairly uniform distribution of ages. As well, due to lower fishing intensities on northern Gulf of St. Lawrence herring (especially in Division 4S), the catches include older age classes compared to southern Gulf catches which historically show peaks at around 4 to 5 years.

The percent agreement levels obtained in this study reflect the relative age-reading difficulty of these particular otoliths. They were from samples with a predominance of older herring. In addition, 150 of the 200 otoliths were from summer catches, and the edges were often difficult to interpret (opaque or hyaline, new growth or none). However, the low CVs and absence of asymmetry show that the levels of age-reading consistency between the two laboratories are acceptable and should have no impact on the assessments of the adjacent herring stocks.

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REFERENCES

Campana, S. E., M. C. Annand, and J. I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124: 131-138.

Campana, S. E. 2001. Accuracy, precision, and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish. Biol. 59: 197-242.

Chang, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39: 1208-1210.

Fishery Biology Program (FBP). 2008. Quality assurance and quality control estimates for the ageing of northwest Atlantic species [website]. Woods Hole (MA): Northeast Fisheries Science Center [Available from: www.nefsc.noaa.gov/fbi/QA-QC/]

Hoenig, J. M., M. J. Morgan, and C. A. Brown. 1995. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52: 364-368.

McQuinn, I. H. 1989. Identification of spring- and autumn spawning herring (*Clupea harengus harengus*) using maturity stages assigned from a gonadosomatic index model. Can. J. Fish. Aquat. Sci. 46: 969-980.

Penttila, J., and L. M. Dery. 1988. Age determination methods for northwest Atlantic species. NOAA Tech. Rep. NMFS-72; 135 p. [Available from: http://www.nefsc.noaa.gov/fbi/age-man.html]

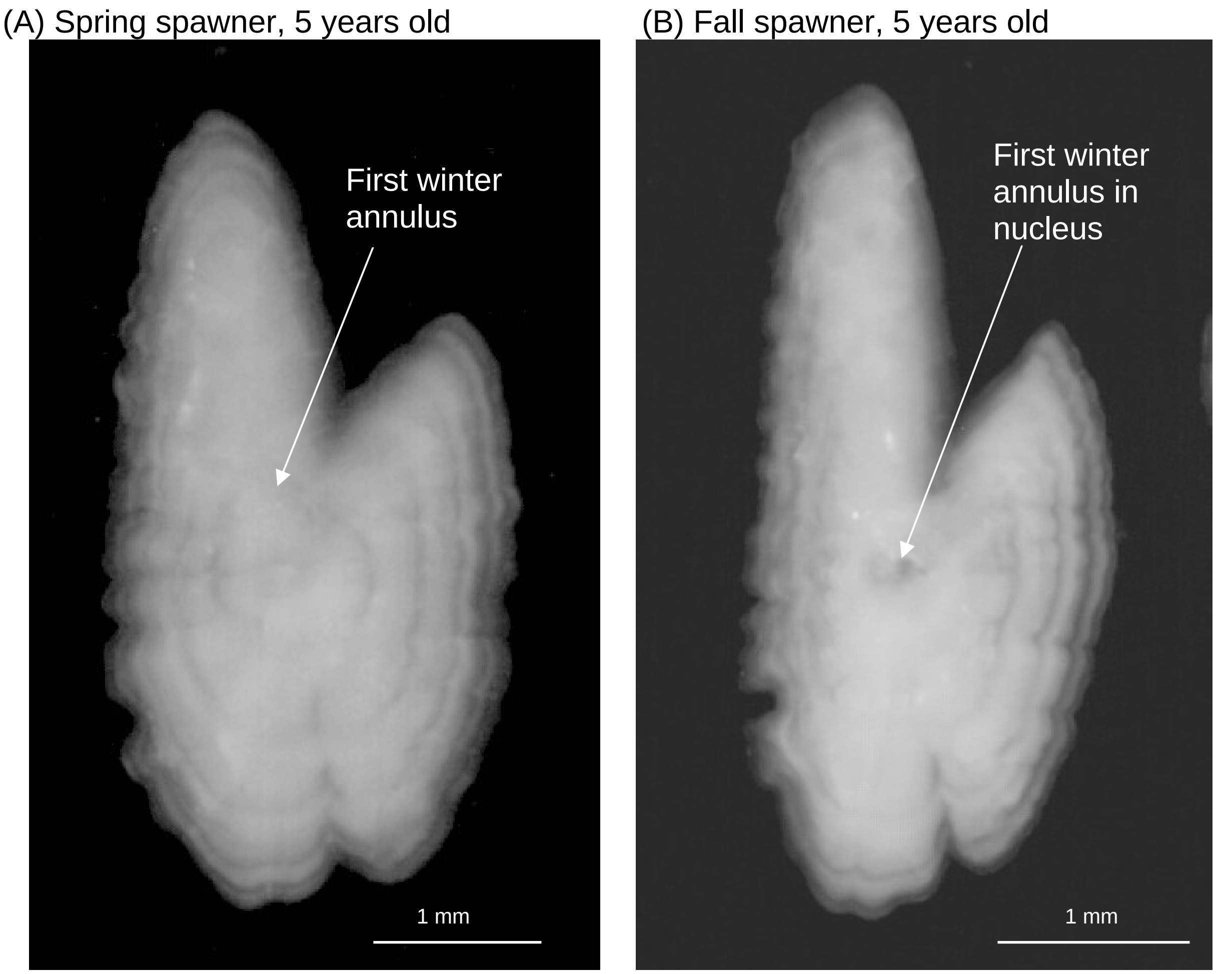
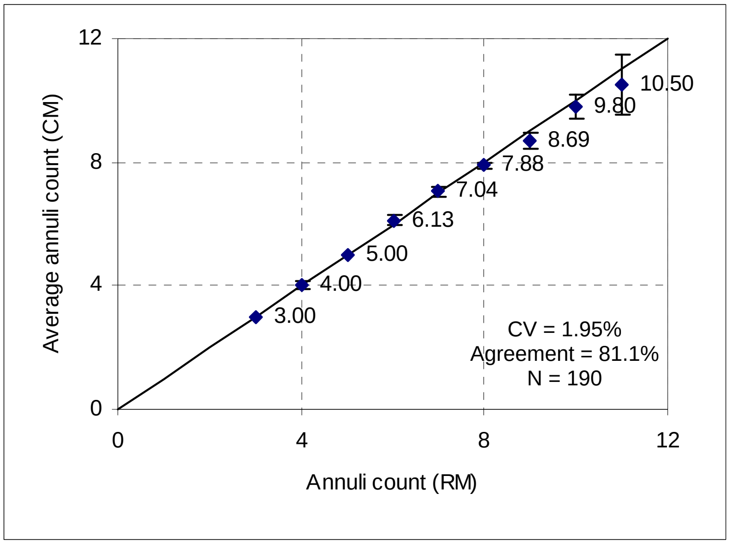


Figure 1. Images of otoliths from a fall-spawner and a spring-spawner herring caught in November in the northern Gulf of St. Lawrence.

A.



B.

Bowker's test	
Chi-sq	15.92
d.f. 10	
P-value	0.10
n/s	

C.

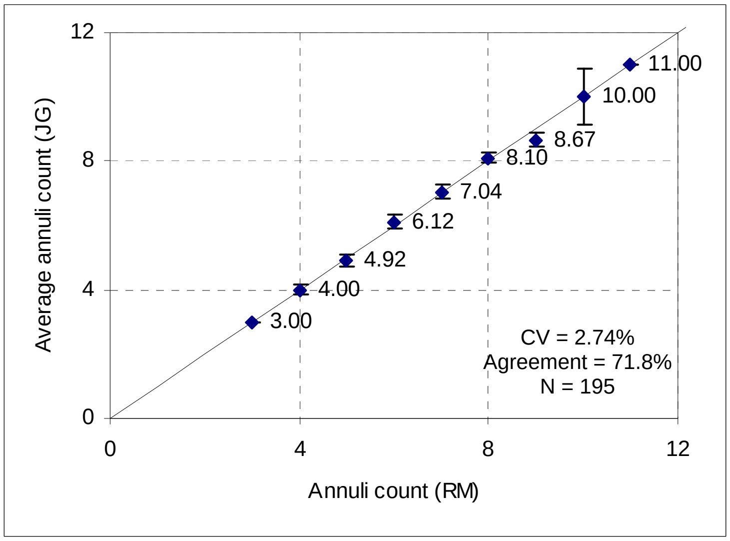
	Annuli count (CM)									
RM	3	4	5	6	7	8	9	10	11+	Total
3	2									2
4	1	19	1							21
5			12							12
6				21	3					24
7				1	22	2				25
8				1	7	50	2			60
9					1	12	20	1	1	35
10							1	4		5
11+						1			5	6
Total	3	19	13	23	33	65	23	5	6	190

D.

Difference					
-3	-2	-1	0	+1	+2
1	2	22	155	9	1

Figure 2. Results of GFC versus 1st MLI herring ageing agreement using fish from 2010 Quebec commercial samples. (A) Age-bias plot; error bars indicate 95% confidence intervals. (B) Bowker's test of symmetry. (C) Age-frequency table. (D) Frequency of differences in annuli counts.

A.



B.

Bowker's test	
Chi-sq	5.53
d.f. 9	
P-value	0.79
n/s	

C.

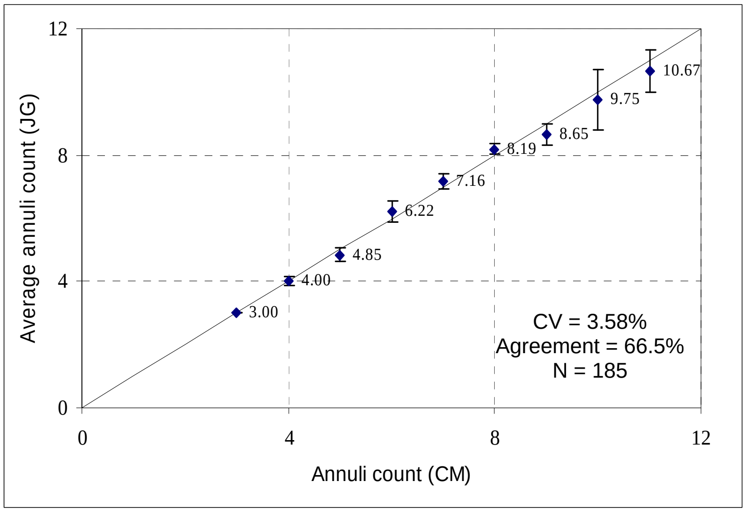
	Annuli count (JG)									
RM	3	4	5	6	7	8	9	10	11+	Total
3	2									2
4	1	19	1							21
5		1	11							12
6			2	19	3	1				25
7				3	18	4				25
8				1	4	44	12			61
9						15	18	3		36
10							2	1	2	5
11+									8	8
Total	3	20	14	23	25	64	32	4	10	195

D.

Difference				
-2	-1	0	+1	+2
1	28	140	25	1

Figure 3. Results of 2nd MLI versus 1st MLI herring ageing agreement using fish from 2010 Quebec commercial samples. (A) Age-bias plot; error bars indicate 95% confidence intervals. (B) Bowker's test of symmetry. (C) Age-frequency table. (D) Frequency of differences in annuli counts.

A.



B.

Bowker's test	
Chi-sq	11.68
d.f. 13	
P-value	0.55
n/s	

C.

CM	Annuli count (JG) 3	4	5	6	7	8	9	10	11+	Total
3	2									2
4	1	18	1							20
5		2	11							13
6			2	17	2	1	1			23
7				4	20	7	1			32
8				2	2	41	16	1		62
9						12	8	2	1	23
10							2	1	1	4
11+							1		5	6
Total	3	20	14	23	24	61	29	4	7	185

D.

Difference					
-2	-1	0	+1	+2	+3
3	25	123	29	4	1

Figure 4. Results of 2nd MLI versus GFC herring ageing agreement using fish from 2010 Quebec commercial samples. (A) Age-bias plot; error bars indicate 95% confidence intervals. (B) Bowker's test of symmetry. (C) Age-frequency table. (D) Frequency of differences in annuli counts.

Appendix 1. Total length (cm) versus annuli count for fall-spawner Atlantic herring aged in the present study by the three otolith readers. (A) RM, the primary MLI reader; (B) CM, the primary GFC reader; (C) JG, the secondary MLI reader. ML is mean total length.

A.

Length (cm)	Annuli count of RM: 3	4	5	6	7	8	9	10	11+	Total
25	1									1
26		1								1
27		2								2
28		5	1							6
29	1	9	1	1						12
30		4	5	3	1	1				14
31			5	6	3	2				16
32				10	9	10	6			35
33				5	8	26	12			51
34					2	14	14	4		34
35					1	6	3	1	1	12
36							2		3	5
37									1	1
38									1	1
39									2	2
Total	2	21	12	25	24	59	37	5	8	193
ML	27.0	28.6	30.2	31.6	32.4	33.1	33.5	34.2	37.0	

B.

Length (cm)	Annuli count of CM: 3	4	5	6	7	8	9	10	11+	Total
25	1									1
26		1								1
27		2								2
28	1	3	2							6
29		10	1							11
30		4	5	1	3	1				14
31			5	6	3	2				16
32				9	9	13	4			35
33				5	11	27	6			49
34				2	1	18	9	4		34
35					2	5	2	1	1	11
36							2		2	4
37									1	1
38									1	1
39									1	1
Total	2	20	13	23	29	66	23	5	6	187
ML	26.5	28.7	30.0	32.0	32.3	33.1	33.7	34.2	36.8	

Appendix 1. Continued.

C.

Length (cm)	Annuli count of JG 3	4	5	6	7	8	9	10	11+	Total
25	1									1
26		1								1
27		2								2
28	1	4	1							6
29	1	8	2		1					12
30		4	5	3	1	1				14
31		1	5	7	2	1				16
32			1	9	7	15	3			35
33				4	9	29	8	1		51
34					3	16	14	1	1	35
35						3	7		2	12
36								2	3	5
37									1	1
38									1	1
39									2	2
Total	2	20	14	23	23	65	32	4	10	194
ML	27.3	28.8	30.2	31.6	32.3	33.0	33.8	34.8	36.5	