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Results of the Atlantic Cod (*Gadus morhua*) Otolith Exchange Between Canada and Portugal

by

A. Ferreira¹, H. Hicks², G. Cossitt², D. Pittman² and C. Hiscock²

¹ Instituto de Investigação das Pescas e do Mar, Av. Brasília 1400 Lisboa, Portugal

² Dep. of Fisheries and Oceans, Science Branch, P.O. Box 5667 St. John's, NF, Canada.

Abstract

An exchange of 101 otoliths of *Gadus morhua* obtained by the Portuguese commercial fleet was conducted between Portugal and Canada in 2004 to verify if the ageing criteria are being applied equally by all readers. A combination of statistical methods and graphical analysis was used to evaluate differences in terms of bias and precision. In general there wasn't detection of bias between age readers and the precision index's calculated indicate a high level of precision. The coefficient of variation presented values under 5%, the average percent error presented values rounding the 3% and the correlation coefficient values above the 0.9. The lowest percent agreement was register for the Portuguese and Canadian 4 age readers (66%) while the highest value was obtained by Canadian 2 vs Canadian 1 and Canadian 1 vs Canadian 4 readers (82% for both comparisons), however with the tolerance level of ± 1 year the percent agreement between age readers was above 90% in all cases. The results of the exchange indicate that in general the ageing criteria are being applied equally by all readers.

Introduction

In Portugal, the Atlantic Cod (*Gadus morhua* Linnaeus, 1758) otoliths collected by the Portuguese commercial fleet operating in NAFO Div. 3NO weren't examine since 1999, dew to the lack of availability from the age readers in the Institute. In 2004 it was possible for a new age reader to analyze the stored otoliths; however it was imperative to assess if the ageing criteria are being applied equally by all readers, for that reason an otolith exchange with Canada was conducted.

The age group estimates based on the interpretation of growth rings in calcified structures (otoliths) has an inherent subjectivity since the growth marks vary in appearance and relative size in different individuals of the same specie. The otolith exchange between Institutes, allows the detection of systematic differences (**bias**) or uniformity (**precision**) of the age groups assigned by independent readers. Of the two referred errors (bias and precision) bias is the more serious one, affecting significantly the interpretation of precision indices (Campana *et al.*, 1995).

This Atlantic Cod otolith exchange was carried out in order to examine differences in age groups estimates among age readers from Portugal and Canada.

Material and Methods

A total of 101 pair of otoliths obtained by the Portuguese commercial fleet in 1999 and 2000 operating in NAFO Div. 3NO were stored dry and sent from Portugal to Canada. The individuals from witch the otoliths were removed ranged in furcal length from 22 to 122 cm and the total weight from 0.092 kg to 32 400 kg.

One otolith from the pair (regardless of being the left or right) was cut with an abrasive blade Superior[®] by its transversal plan, passing the nucleus. Whenever there were doubts about the age group the respective pair was also cut.

In Portugal, Ana Ferreira (**PRT**) examined the otoliths with a binocular microscope (Olympus[®] SZX12) under transmitted light (fiber optic illuminator Schott[®] KL1500Z) at magnifications of (10–20x) with the help of the device described by Bedford in 1964. To clarify the deposition pattern of the growth rings, a solution of 1:1 Ethanol and Glycerine was used in the reading surface of the otolith.

In Canada, Harry Hicks (**CAN 1**), Gus Cossitt (**CAN 2**), Dwayne Pittman (**CAN 3**) and Charlie Hiscock (**CAN 4**) examined the otoliths with a dissecting binocular microscope (Nikon[®]) under transmitted light (fiber optic illuminator) at magnifications of 14x. A scalped blade was used to shade the top of the cut section and to prevent any glare from the surface of the cut sections alcohol or "Kodak Photo - Flo 200 solution" was applied to the top of the cut section.

A set of statistical tests and graphical methods were applied to compare the bias and the precision between readers.

To detect **bias** between readers the nonparametric Wilcoxon matched-pairs rank test ($\alpha = 0, 05$) was used, since the presupposes of normality (Shapiro – Wilks test) and homogeneity of variances (Levene's test) aren't verified, to detect significant differences between the age groups assigned by the readers. Age bias graphs and graphs representing differences in age estimates between paired readers are presented to visual detect the systematic age differences between readers.

To analyze **precision** between readers the coefficient of variation (CV), average percent error (APE) and percent agreement were calculated. Graphs of coefficient of variations plus percent agreement by age group and graphs of percent agreement to different levels of tolerance in differences between age group estimates were also analyzed to visually detect precision between readers. The correlation coefficient of Spearman (Zar, 1996) is given to evaluate the fraction of variation explained by the linear relationship between readers.

Results

Regarding the **bias** between age readers, the Wilcoxon test (Table 1) reveals that there aren't significant differences between the average age group estimated by the different readers for the otolith collection exchanged.

The graphical analysis performed to detect bias (panels A and B – Fig. 1 to 10) supports this test result, as the age bias graphs (panels A – Fig. 1 to 10) don't show any significant deviation to the solid line (1:1 equivalence), with the exception of the Portuguese vs Canadian 4 (panel A – Fig. 10) where the Portuguese reader tends to present higher age groups than the Canadian one after age group 12.

The maximum differences in the age group estimates are assigned to the Portuguese reader with all the Canadian readers (panels B – Fig. 4, 7, 9, and 10) but only after the age group 11 where the number of otoliths per age group is small.

Concerning the **precision** of the age group estimates, the correlation coefficient (Table 2) indicates that a high fraction of variation in the age group estimates is explained by the linear relationship between readers.

The Portuguese reader is the one that register the highest values of coefficient of variation with all the Canadian readers however the 5% value is never reached (Table 2). The same result is obtained with the average percent error, where the average 3% value is obtained for the Portuguese reader with all the Canadian. The minimum values obtained for the referred indices (CV and APE) concern the comparison of the Canadian readers 1 and 2 (Table 2).

The maximum percent agreement (with ± 0 years tolerance) between readers (82%) is achieved by Canadian 2 vs Canadian 1 and Canadian 2 vs Canadian 4. The percent agreement of the Portuguese reader with the Canadian readers is close to the maximum achieved and averages the 70% (Table 2).

The graphical analyses of precision (panels C and D – Fig. 1 to 10) also indicate high levels of precision between readers. The graphs showing the coefficient of variation and percent agreement by age group illustrate high precision levels until age group 11 (panels C – Fig. 1 to 10) and the graphs of percent agreement to different levels of tolerance (panels D – Fig. 1 to 10), show that with the tolerance of ± 1 year the percent agreement of all readers is above 90%.

Conclusion

There wasn't significant bias between any of the age readers and the major differences in age group estimates occurred in the oldest age groups were the number of otoliths per age group was small.

Despite the fact that the Portuguese age reader is the one with the smallest precision in the group of age readers, the Index values and statistic analyses performed indicate that the precision of this age reader is very close to the one obtained for the other age readers, so this source of error is not preoccupant to the age group estimates of the Portuguese reader.

Acknowledgements

To Dr. Ricardo Alpoim, for the revision of the manuscript and teaching of the age – determination techniques. This study was supported by the European Commission (DG XIV, Program for the collection of data in fisheries sector) and IPIMAR.

References

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Tab. 1: Statistical test for the detection of bias between readers

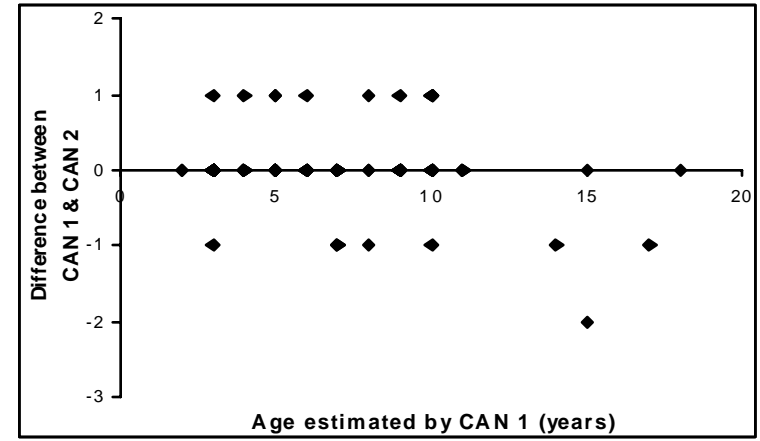
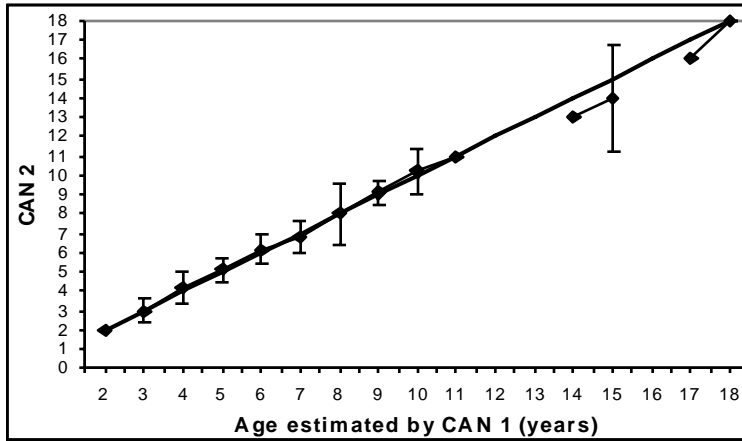
	CAN 1	CAN 1	CAN 1	CAN 1	CAN 2	CAN 2	CAN 2	CAN 3	CAN 3	CAN 4
	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>
	CAN 2	CAN 3	CAN 4	PRT	CAN 3	CAN 4	PRT	CAN 4	PRT	PRT
	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)
Wilcoxon test										
T	81	189	67	229	100	65	180	217	139	212
Z	0,196	0,330	1,147	0,664	0,187	0,893	1,080	1,135	0,632	1,462
P	0,845	0,741	0,251	0,507	0,852	0,372	0,280	0,257	0,527	0,144

Tab. 2: Measures of precision between readers.

	CAN 1	CAN 1	CAN 1	CAN 1	CAN 2	CAN 2	CAN 2	CAN 3	CAN 3	CAN 4
	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>	<i>vs</i>
	CAN 2	CAN 3	CAN 4	PRT	CAN 3	CAN 4	PRT	CAN 4	PRT	PRT
Statistic or index	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)	(N = 101)
Correlation coefficient (r_s)	0,991	0,983	0,992	0,978	0,988	0,990	0,978	0,984	0,973	0,980
Coefficient of variation (%)^a	1,931	3,315	2,054	4,234	2,663	2,238	4,430	2,884	4,737	4,485
Average percent error^b	1,365	2,344	1,452	2,994	1,883	1,583	3,133	2,039	3,350	3,171
Percent agreement	82	72	81	68	80	82	70	75	67	66

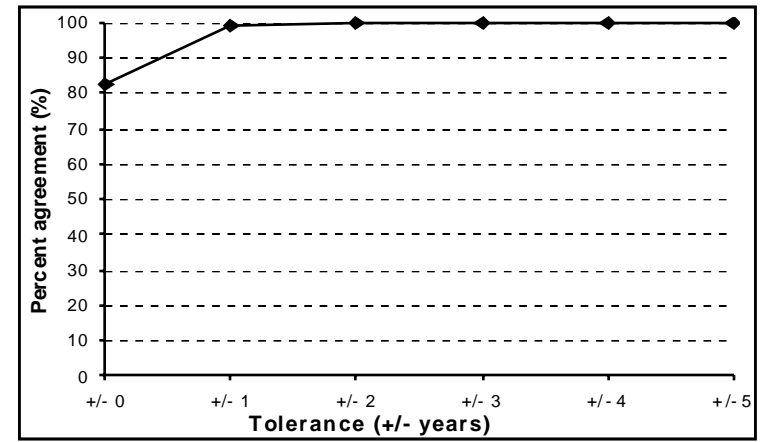
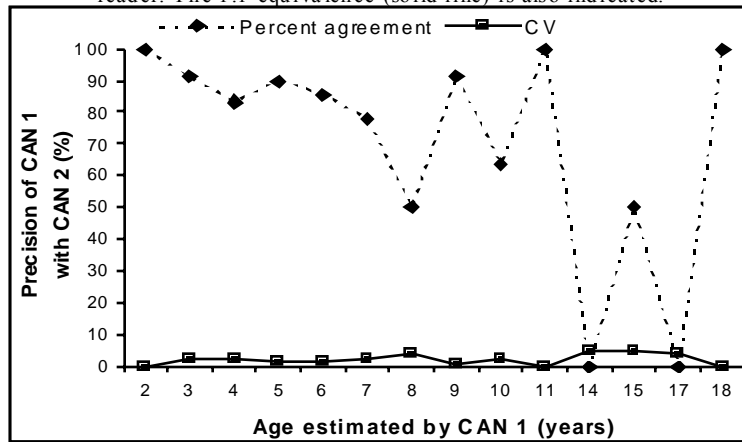
^a Chang (1982)^b Beamish and Fournier (1981)

Fig. 1: Graphical methods for comparison between readers: CAN 1 vs CAN 2



A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.

B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish

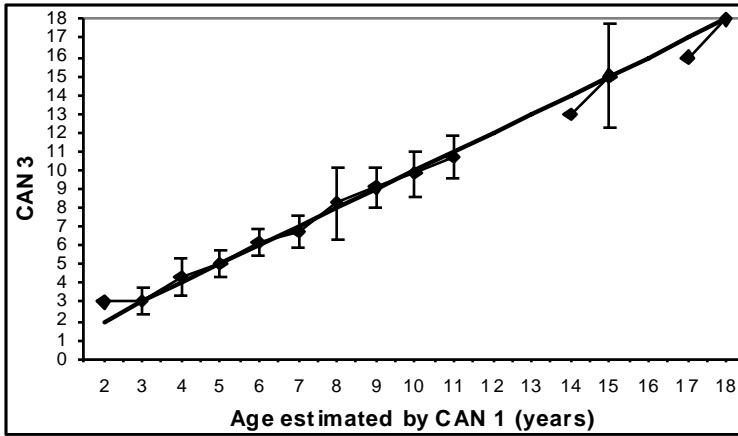


C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.

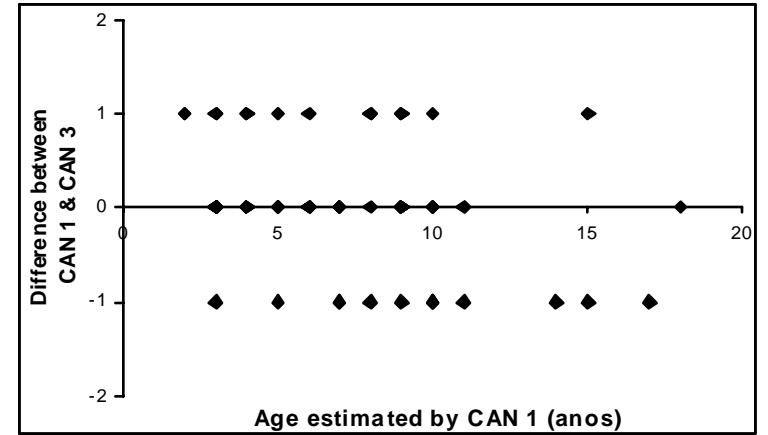
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	2	22	6	20	7	9	4	12	11	3	0	0	1	2	0	1	1

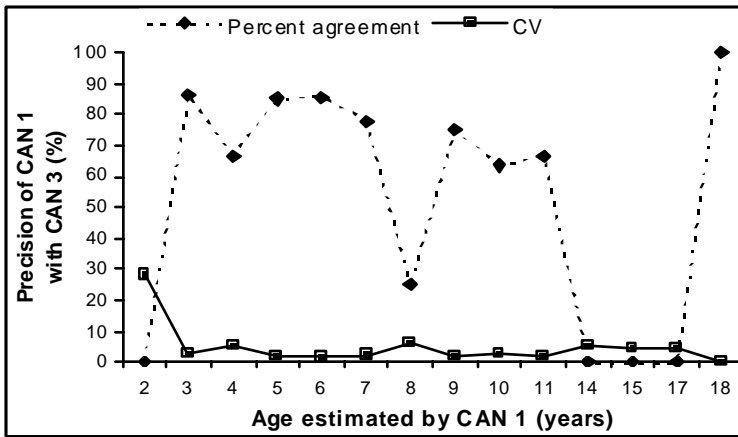
Fig. 2: Graphical methods for comparison between readers: CAN 1 vs CAN 3



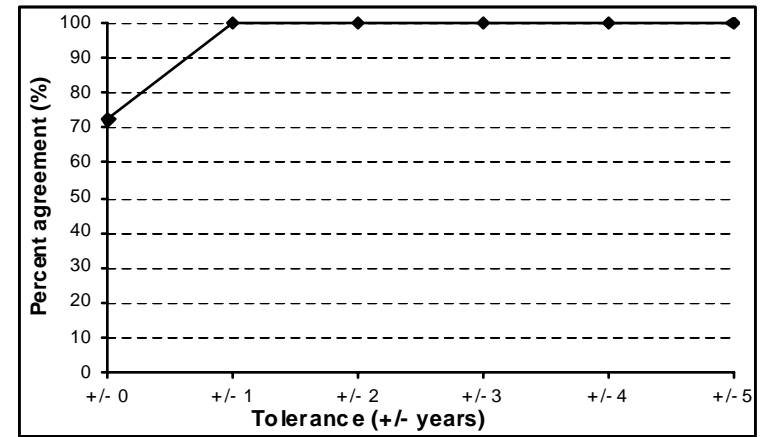
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



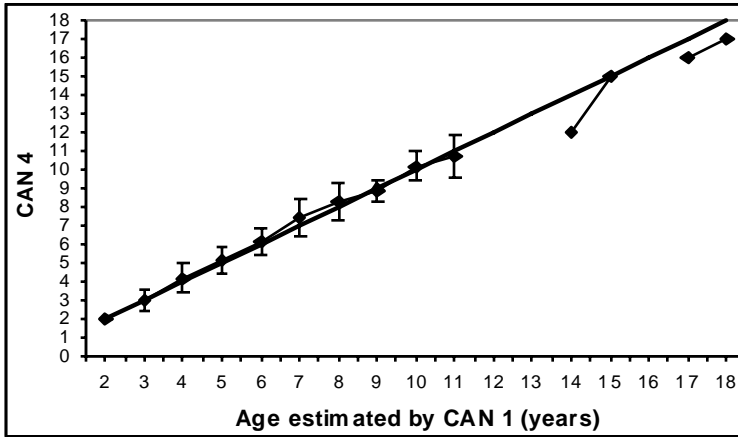
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



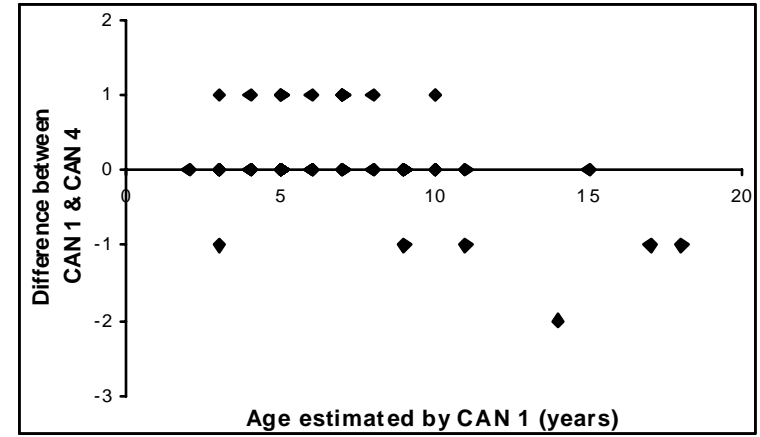
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	2	22	6	20	7	9	4	12	11	3	0	0	1	2	0	1	1

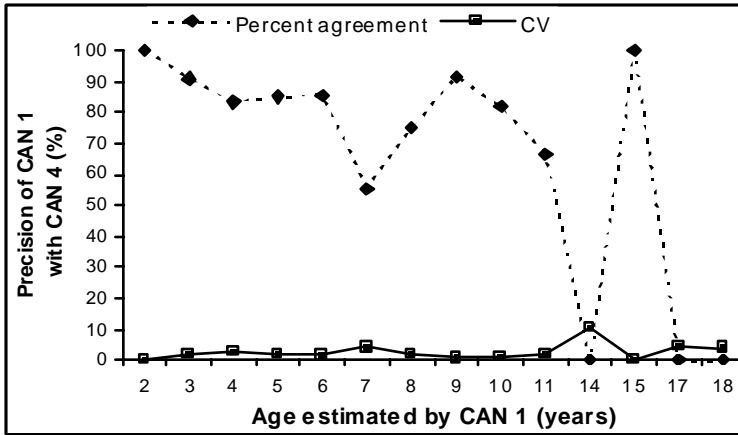
Fig. 3: Graphical methods for comparison between readers: CAN 1 vs CAN 4



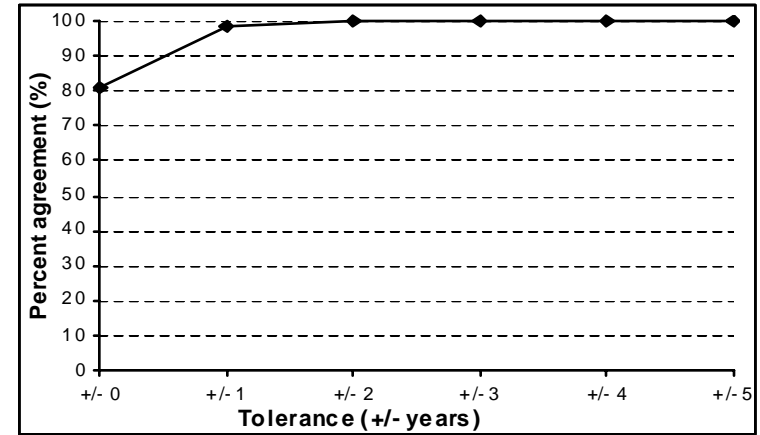
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



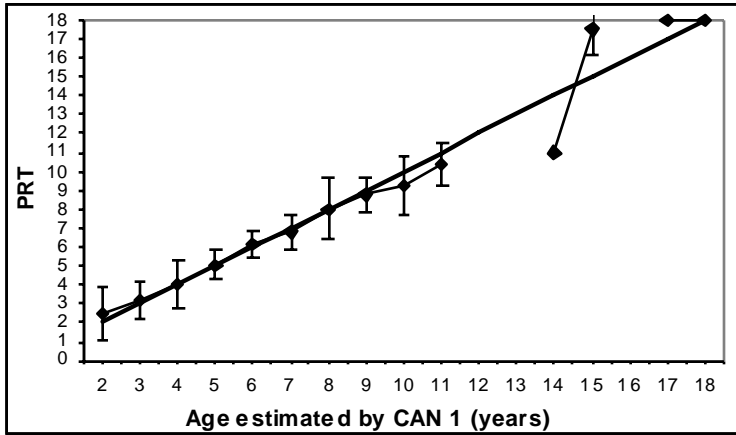
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



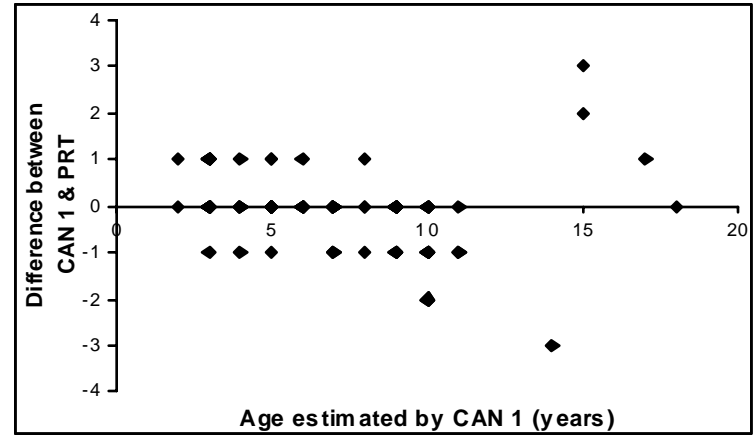
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	2	22	6	20	7	9	4	12	11	3	0	0	1	2	0	1	1

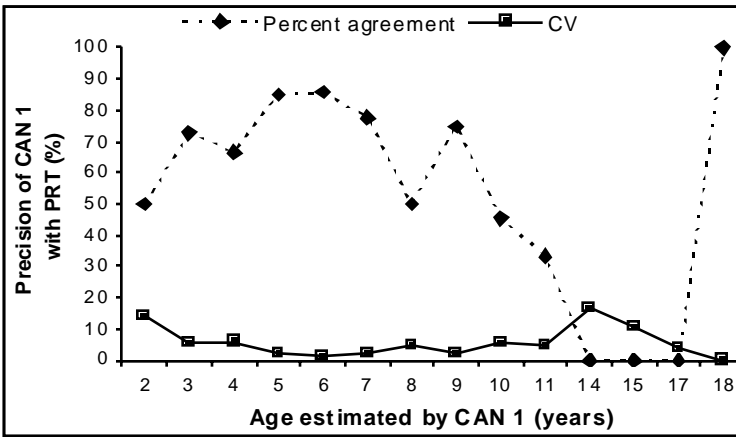
Fig. 4: Graphical methods for comparison between readers: CAN 1 vs PRT



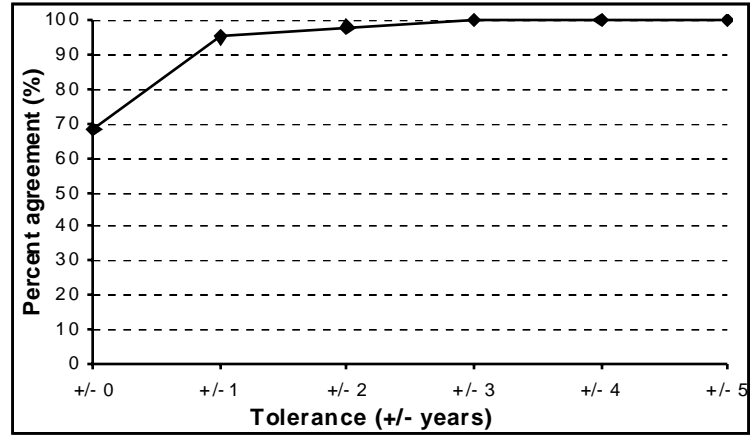
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



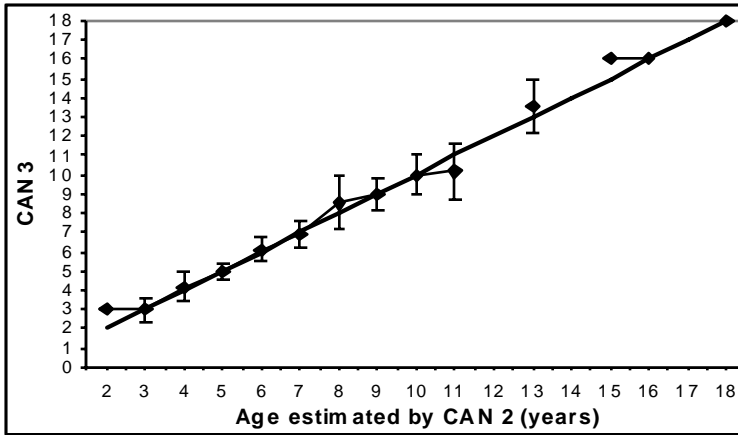
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



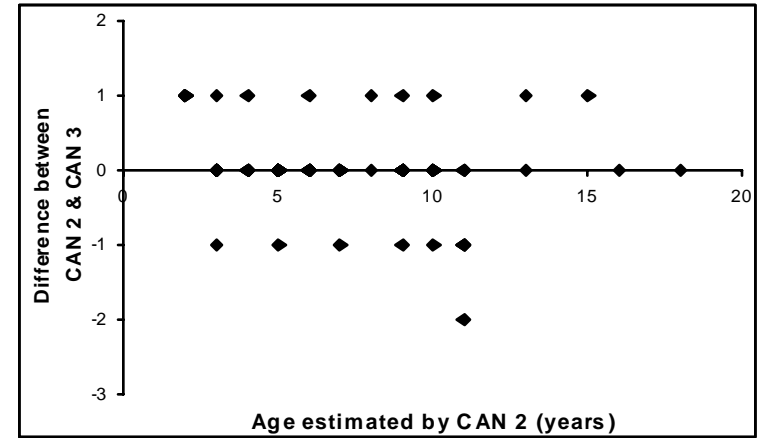
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	2	22	6	20	7	9	4	12	11	3	0	0	1	2	0	1	1

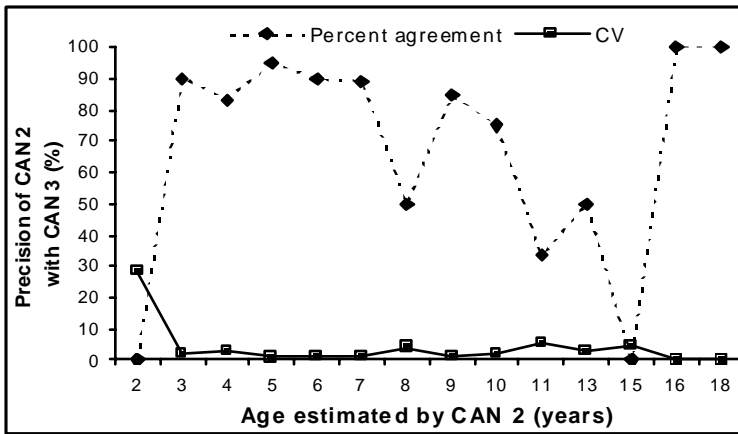
Fig. 5: Graphical methods for comparison between readers: CAN 2 vs CAN 3



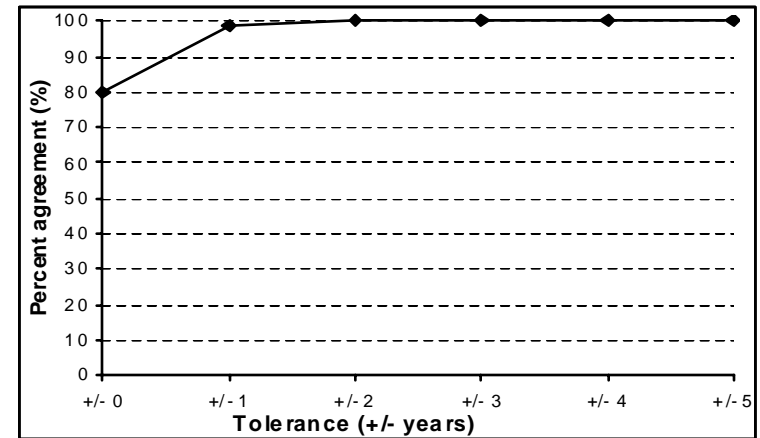
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



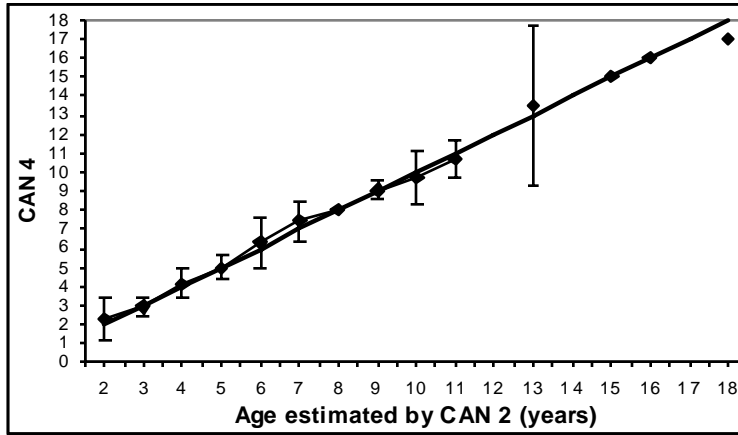
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



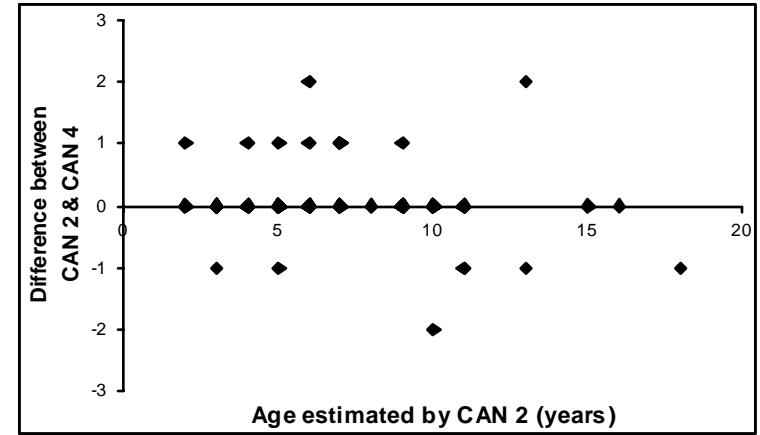
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	3	20	6	19	10	9	2	13	8	6	0	2	0	1	1	0	1

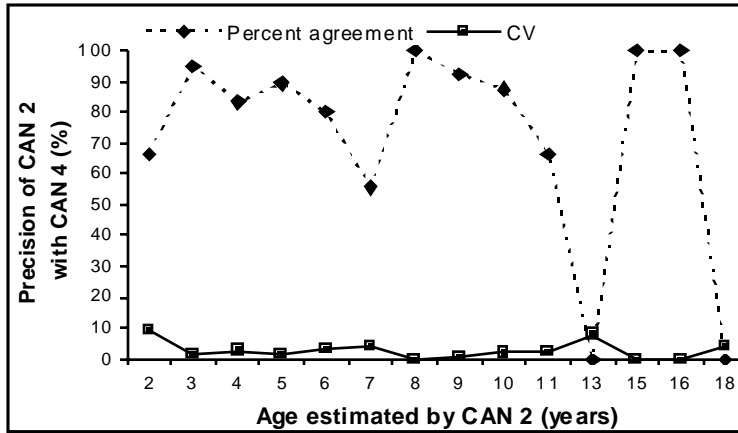
Fig. 6: Graphical methods for comparison between readers: CAN 2 vs CAN 4



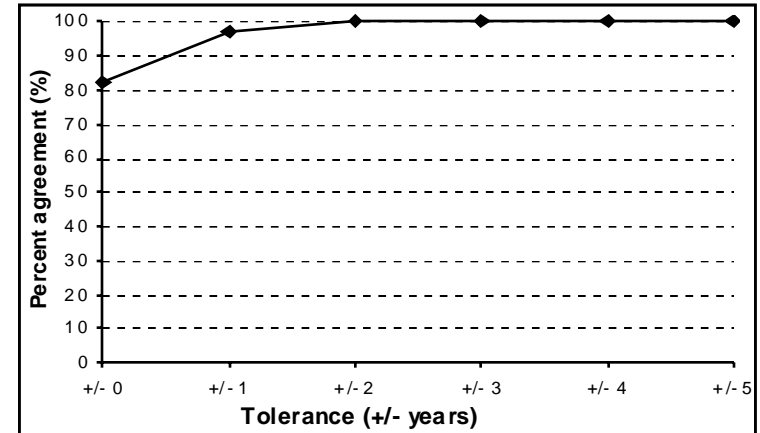
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



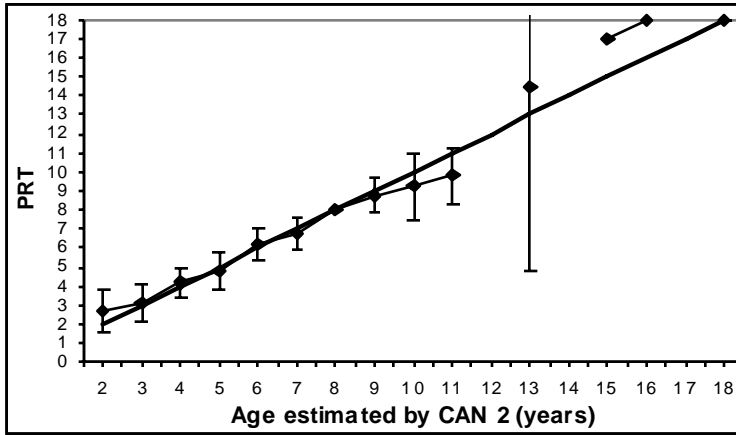
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



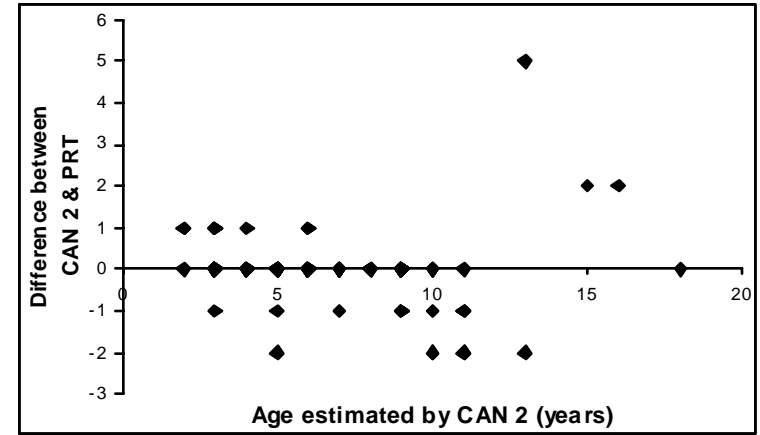
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	3	20	6	19	10	9	2	13	8	6	0	2	0	1	1	0	1

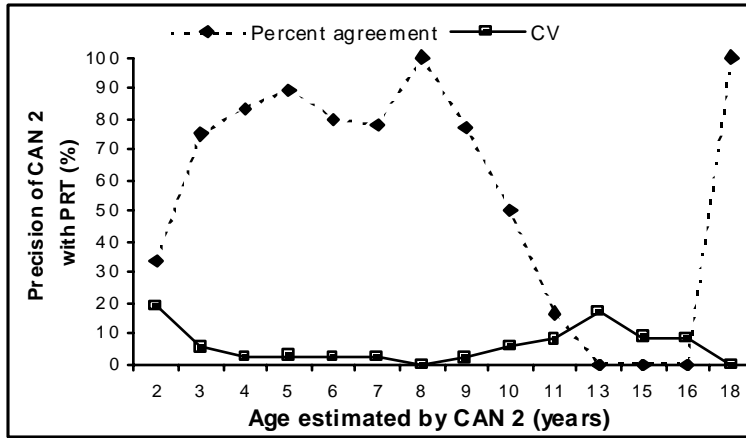
Fig. 7: Graphical methods for comparison between readers: CAN 2 vs PRT



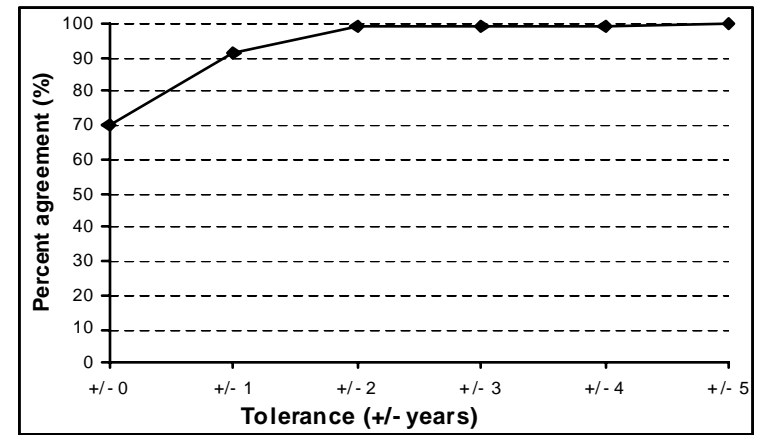
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



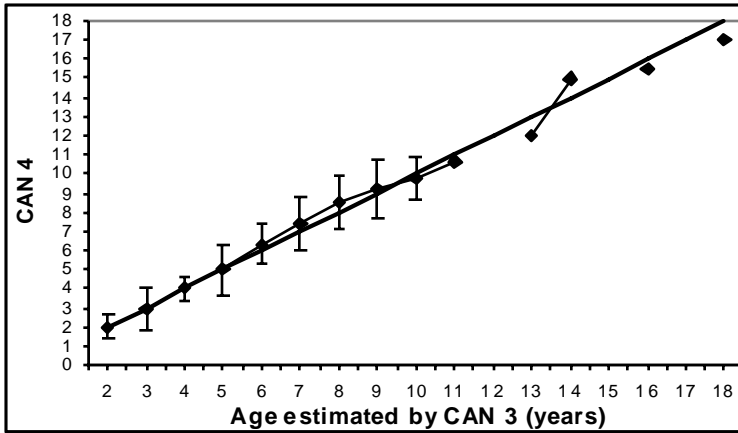
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



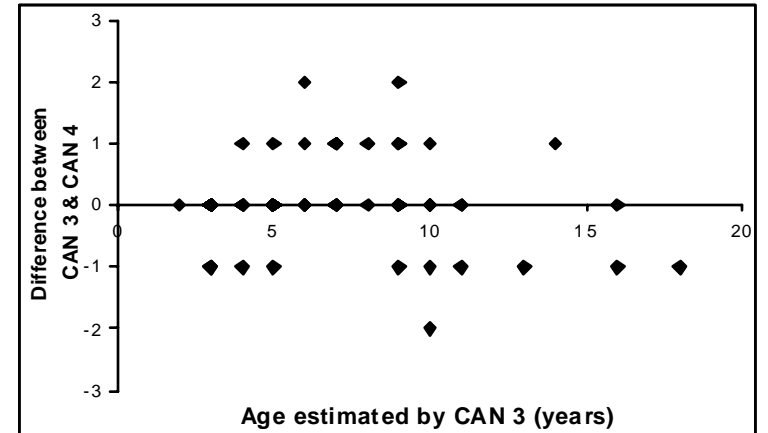
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	3	20	6	19	10	9	2	13	8	6	0	2	0	1	1	0	1

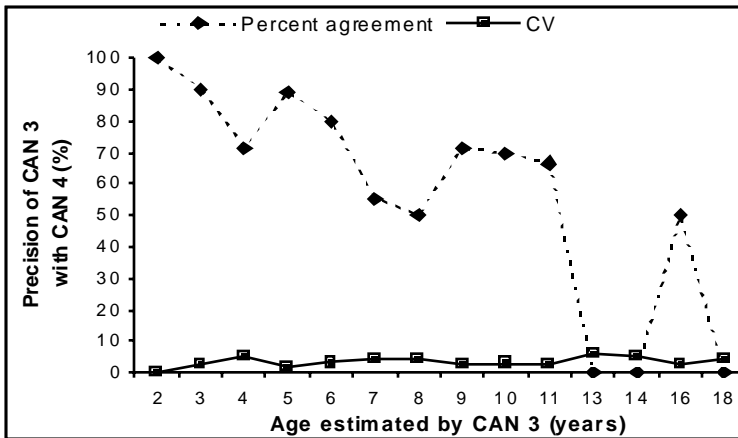
Fig. 8: Graphical methods for comparison between readers: CAN 3 vs CAN 4



A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



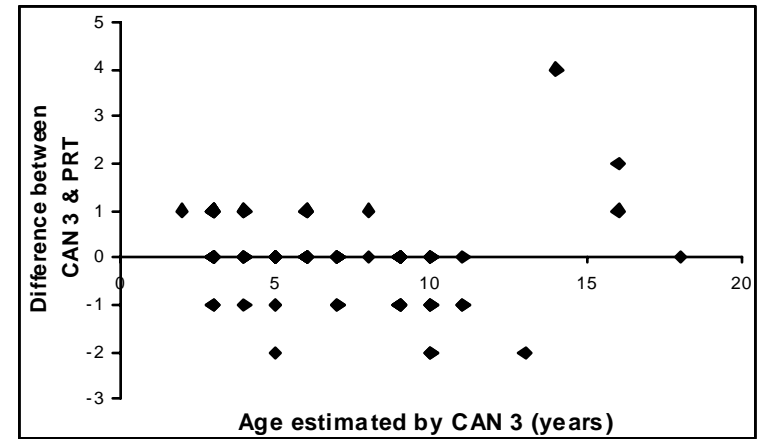
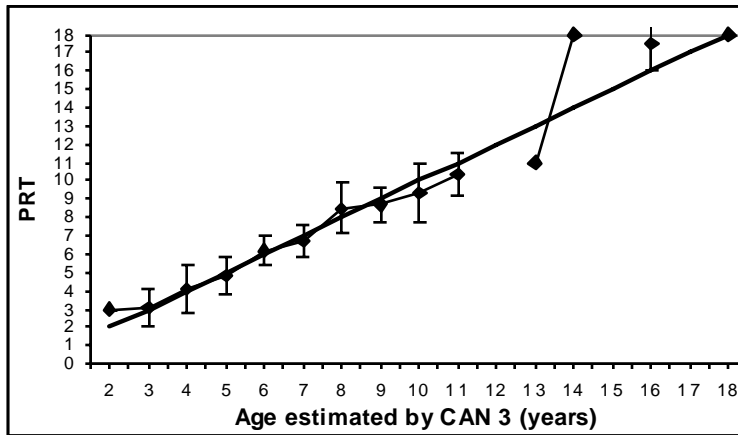
C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

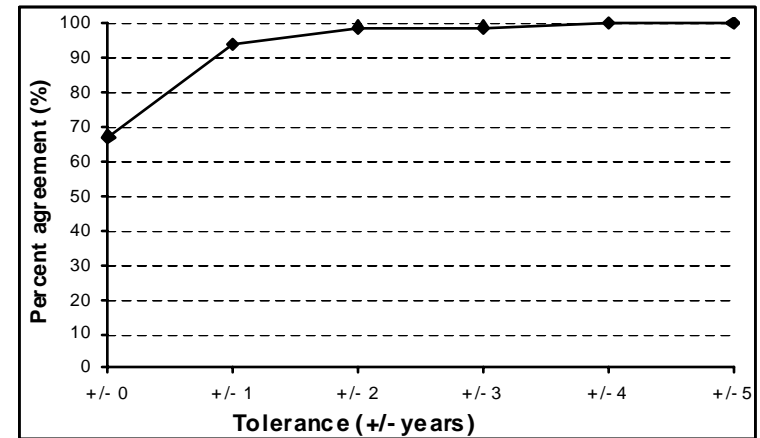
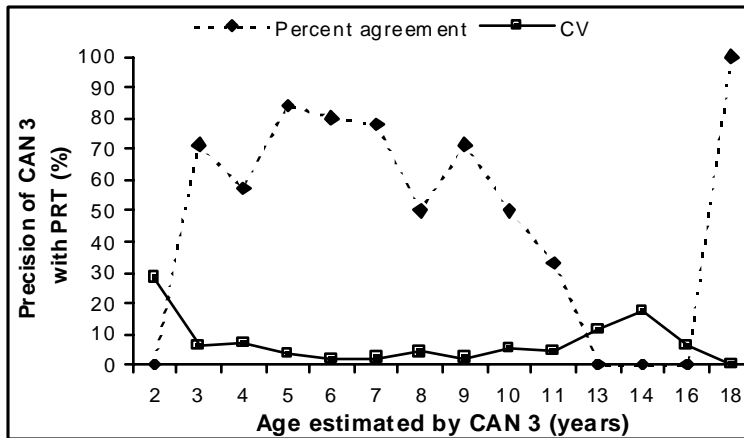
Group age estimated by CAN 3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	1	21	7	19	10	9	2	14	10	3	0	1	1	0	2	0	1

Fig. 9: Graphical methods for comparison between readers: CAN 3 vs PRT



A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.

B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish

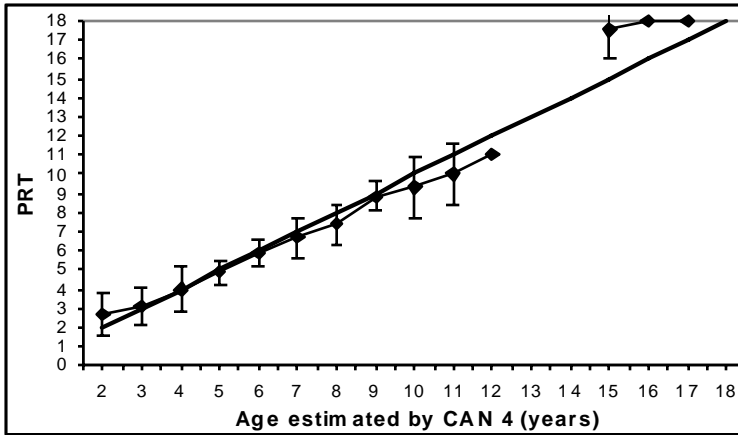


C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.

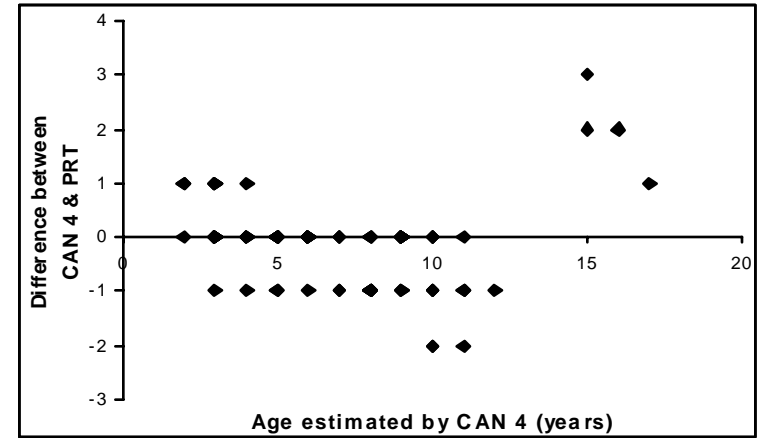
D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 3	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	1	21	7	19	10	9	2	14	10	3	0	1	1	0	2	0	1

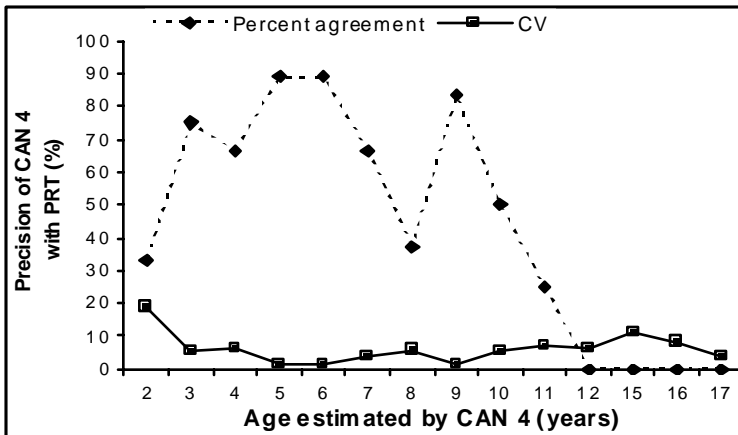
Fig. 10: Graphical methods for comparison between readers: CAN 4 vs PRT



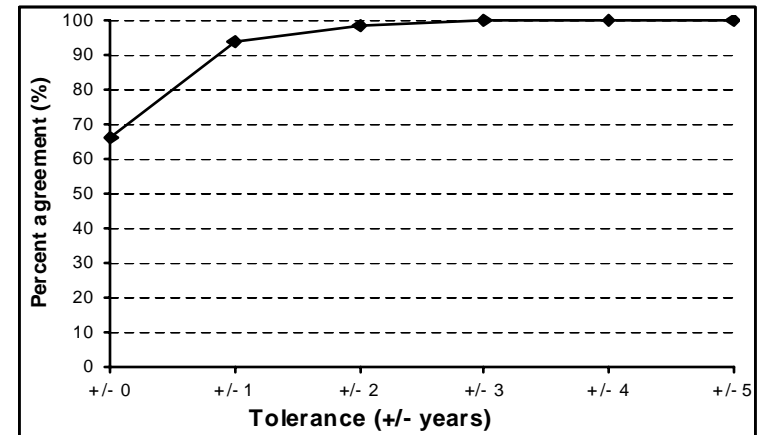
A- Age bias graphs. Each error bar represents the 95% confidence interval about the mean age assigned by one reader for all fish aged by a second reader. The 1:1 equivalence (solid line) is also indicated.



B- Differences (years) in age estimates between paired age readers. Each point represents one or more observations of an individual fish



C- Coefficient of variation (CV) and percent agreement for two of the pairwise age comparisons.



D- Percent agreement in reader's comparisons for a tolerance level of +/- 0 to +/- 5 years applied to all age groups assigned by CAN 1.

Group age estimated by CAN 4	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
n	3	20	6	18	9	6	8	12	10	4	1	0	0	2	1	1	0