The use of Otolith Reference Collections to Determine Ageing Precision of Red Grouper *(Epinephelus morio)* Between Fisheries Laboratories

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SEDAR 42-DW-13

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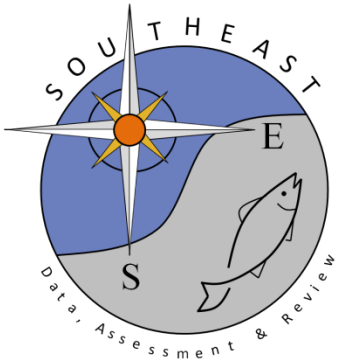

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Introduction

Species-specific age data are required for the Southeast Data Assessment Review (SEDAR) process. SEDAR 42 will begin in November 2014 to review the red grouper, *Epinephelus morio,* stock assessment issues with age data provided by the National Marine Fisheries, Panama City Laboratory (PCLAB) and the Florida Fish and Wildlife Conservation Commission/Fish and Wildlife Research Institute (FWRI). Quality age data for fisheries assessments are critical needs for understanding the relationships between fisheries and fisheries management. To insure ageing standards of a given species are met, red grouper otolith reference collections were developed independently by the PCLAB and FWRI laboratories to monitor precision between 11 otolith readers. These 11 readers were responsible for completing age determination of red grouper collected in 1979 – 2013 that will be used by SEDAR 42.

There are three important components to this study: (1): to monitor in-house reader precision, (2) to compare reader precision between laboratories using different reference collections, and (3) to insure that the age data being generated for stock assessment needs meets quality standards. In previous studies (e.g. Allman, 2004) results proved that the use of an otolith reference collection is a fitting mechanism for validation of ageing methods. An otolith reference collection's primary role is to gauge ageing consistency for the short-term and long-term duration of a reader's term (Campana, 2001). The objectives of this report are to illustrate the precision results from the reference collections and ageing matrices of contributing readers from the two laboratories for red grouper.

Methods

Training readers

The sagittal otolith as described by Moe (1969) was used by both laboratories to create their respective red grouper reference collections. Before reading the reference collections a training set that consisted of sectioned and whole sagittae ototliths was developed and read by the PCLAB readers. FWRI does not have a training set but did review the PCLAB training set in digital format. These training set otoliths represent the variability in edge types and growth incremental patterns typically found in red grouper otoliths. L. Lombardi (PCLAB) is the only

reader that has previous experience ageing red grouper and was designated as the primary reader at the PCLAB. Two new readers, E. Crow and C. Fioramonti, who during the interim of a previous report (Palmer et al., 2006) describing ageing precision results of readers of red grouper at the PCLAB, were trained to age red grouper. Per the FWRI laboratory, J. Carroll was the primary reader. Two additional readers at the FWRI laboratory, K. Cook, and K. Wolfgang were also trained to age red grouper. All FWRI readers were 'self-taught' by J. Carroll using only sectioned samples using the methods as described by Burgos et al. (2007).

Reference collections

The PCLAB reference collection is composed of whole sagittal otoliths (n=204) from years 2002 - 2005 while the FWRI reference collection is composed of thin sectioned sagittal otoliths (n=200) representing years 2009 – 2013. Red grouper have mainly been aged in preparation for SEDAR 42 by multiple readers (7) from the PCLAB (2001-2013) and recently (2013) FWRI (Table 1). Quarterly distributions of the reference collections along with SEDAR 42 data shows samples and the reference collection were evenly distributed from throughout the year (Figure 1a). Fork lengths from the two reference collections ranged from 354mm - 860mm (PCLAB) and 132mm – 860mm (FWRI) (Figure 1b). In both reference collections a variable representation of otoliths was necessary to insure the collection was composed of easy, hard, and difficult to read otoliths as noted by Campana (2001).

Otolith interpretation

Reference collections from both laboratories were read by all readers (PCLAB n=4, FWRI n=3) at both laboratories using a stereo microscope with magnifications between 5.625x and 150x. Annuli were defined as opaque bands, and readers recorded the number of annuli and edge type (Lombardi-Carlson et-al., 2002). All readers of the reference collections were provided with only a vial number (whole otoliths) or slide number (sectioned otoliths) and no meristic data were made available. Age data (band count, edge type) was entered into a Microsoft EXCEL® spreadsheet for analysis.

The edge type, band count, and capture date were used to calculate the annual age of a fish based on calendar year (Jerald, 1983). Three different classifications for edge types were used to determine if annual ages were advanced one year. Otoliths with a complete opaque zone on the edge of the otolith were classified as edge type 2. The number of annuli and the annual age are equivalent for edge type 2. Red grouper experiencing new growth exhibit varying degrees of translucent growth zones on the edge of the otolith. If translucent growth was less than one-half complete, the otolith was classified as edge type 4, and the number of annuli was the same as the annual age. Otoliths with an edge type 6 had a complete translucent zone on the edge. Fish captured prior to July 1st with an edge type 6 were advanced one year. See Table 2 for the criteria for advancing ages..

Precision

Three indices of precision were used to determine the level of overall accuracy among readers from both laboratories of their respective reference collections, overall among readers from the other laboratories' reference collection, and overall among all readers of each laboratory's reference collection. Overall average percent error (APE), coefficient of variation (CV), and precision (D) were calculated (Kimura and Anderl, 2005; Campana, 2001) for each laboratory's readings including one historical (1991-2001) pair reading from the PCLAB; L. Lombardi – J. Mikulas. This historical reading of whole sagittae (n=3,462) was composed of red grouper ages from 3 – 29 years.

Results and Discussion

Although the PCLAB readers have been reading red grouper otoliths for many years, every year or prior to ageing red grouper the training set and reference collection was re-read by the respective readers. There were no indices of precision calculated from the individual readings of the training set. The PCLAB reference collection was composed of ages 3 - 20 according to the primary reader L. Lombardi and the FWRI reference collection included fish that were from 0 to 16 years old per the primary reader J. Carroll (Figure 1c). The age range

from both reference collections provided a respectable representation of the age range (0 to 29 years) of SEDAR 42 age data (Figure 1c).

Overall reader agreement values show high precision between all seven groupings of readers (Table 3). Precision values were slightly elevated for the PCLAB readings of the FWRI reference collection most likely due to the PCLAB's protocols for ageing red grouper using only whole otoliths. Standard deviation plots of the overall readings (Figure 2a) show no significant bias up to age 12. It was suggested that this increase in standard deviation of the overall reader agreement after age 12 was a result of a low sample size of older fish in the reference collections (Figure 1c). In addition, the historical paired reading of L. Lombardi and J. Mikulas yielded < 5% (n = 165) of fish aged greater than 12 years old. The overall average of all readings for all time periods (PCLAB laboratory readings) and the initial overall reading of the FWRI reference collection reflects high precision for ages 3 through 12 (Figure 2b). Thus, we suggest that the use of a reference collection is a valuable tool to monitor precision between readers and laboratories. Our overall precision results from the two separate reference collections from both laboratories and one historical paired reading illustrate that the age determination of each laboratory's readers were consistent with previous PCLAB precision values. In addition, FWRI red grouper age date provided to SEDAR 42 is a viable source of data based on the high precision of FWRI readers.

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Table 1. Multiple readers contributed red grouper ages to SEDAR 42. Listed are the percentages of age contribution by reader and by year (1979-2013). NMFS PC – National Marine Fisheries Service, Panama City Laboratory; FWRI – FL FWCC/Fish and Wildlife Research Institute.

Year	L. Lombardi NMFS PC	C. Palmer NMFS PC	C. Gardner NMFS PC	R. Farsky NMFS PC	C. Fioramonti NMFS PC	E. Crow NMFS PC	FWRI
1979	100%						
1980	100%						
1981	100%						
1985	100%						
1986	100%						
1987	100%						
1988	100%						
1989	100%						
1991	100%						
1992	100%						
1993	100%						
1994	100%						
1995	100%						
1996	100%						
1997	100%						
1998	100%						
1999	100%						
2000	100%						
2001	100%						
2002	100%						
2003	10%	90%					
2004	17%		83%				
2005	10%			90%			
2006	9%	86%					5%
2007	14%				73%		12%
2008	34%	29%			32%		5%
2009	2%	46%			45%		6%
2010	5%				68%		27%
2011	3%				33%	51%	13%
2012	4%					77%	18%
2013	4%					69%	27%

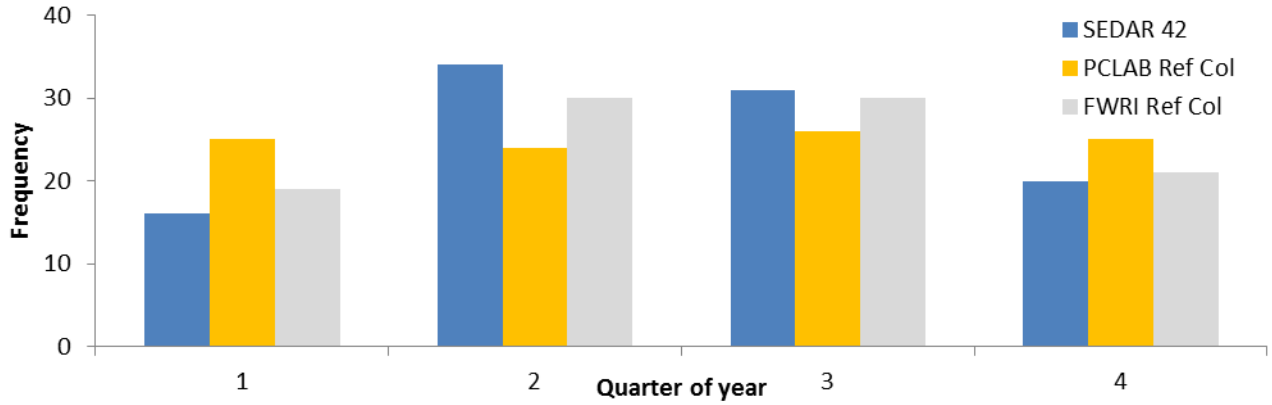
Table 2. Criteria for advancing ages

Collection date	Edge type	Advance annulus count
January 1 – June 30	2,4	0
January 1 – June 30	6	+1
July 1 – December 31	2,4,6	0

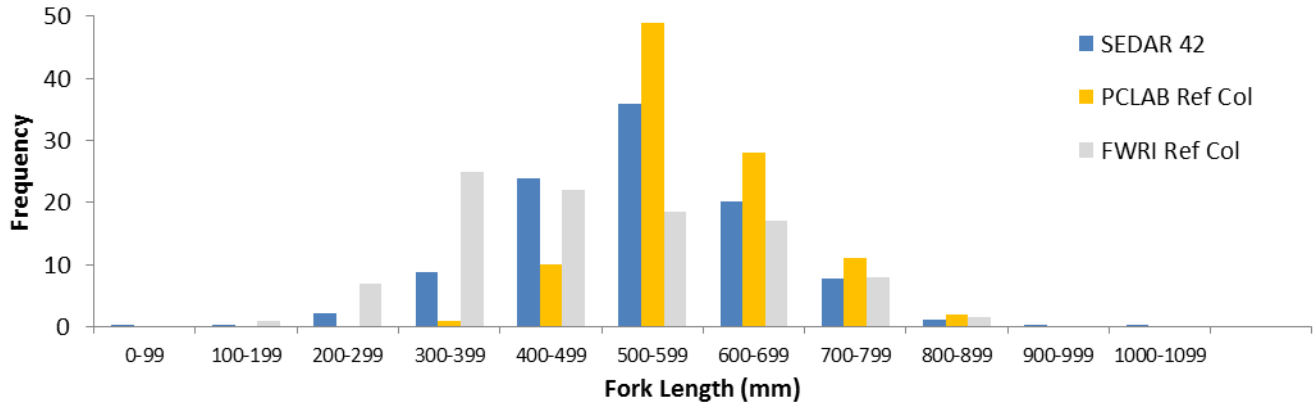
Table 3. Overall precision values for NMFS Panama City Laboratory (PCLAB - L. Lombardi, J. Mikulas, C. Gardner, C. Palmer, B. Farsky, E. Crow, amd C. Firoamonti) and the FL FWCC/Fish and Wildlife Research Institute (FWRI – J. Carroll, K. Cook, and K. Wolfgang).

Sample years	Reader pair	APE	CV	D
1991-2001	L. Lombardi - J. Mikulas (historical reading)	3.38	4.77	3.38
2002-2005	PCLAB reference collection read by PCLAB	3.45	4.53	2.27
2006-2013	PCALB reference collection read by PCLAB	3.21	4.23	2.12
2006-2013	PCLAB and FWRI overall readings of the PCLAB reference collection	3.62	4.87	2.44
2006-2013	PCLAB and FWRI overall readings of the FWRI reference collection	5.10	6.50	3.25

(a.)



(b.)



(c.)

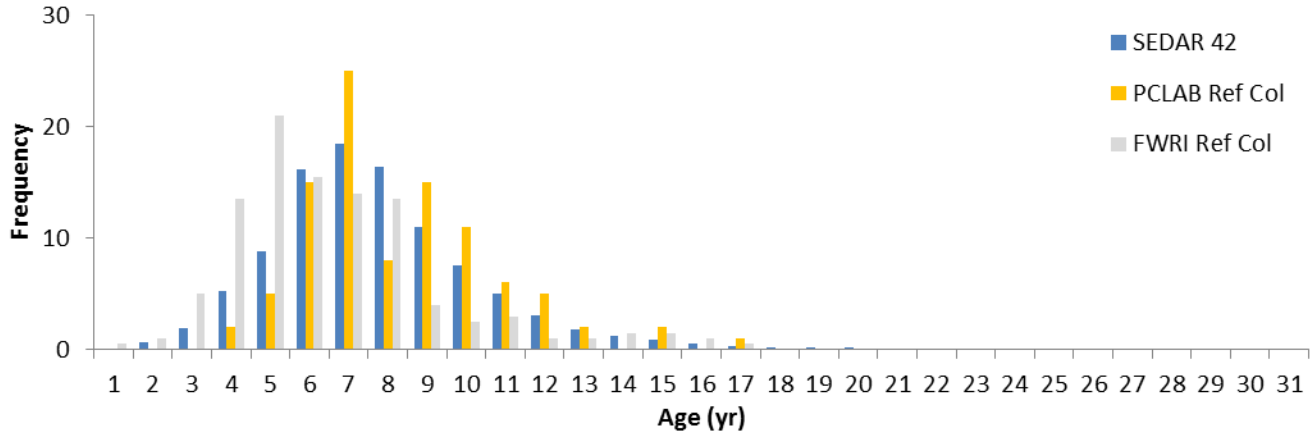


Figure 1. Comparison of the (a) quarterly distribution, (b) length and (c) age frequency of SEDAR 42 data, NMFS Panama City Laboratory reference collection (PCLAB Ref Col), and FL FWCC/Fish and Wildlife Research Institute reference collection (FWRI Ref Col).

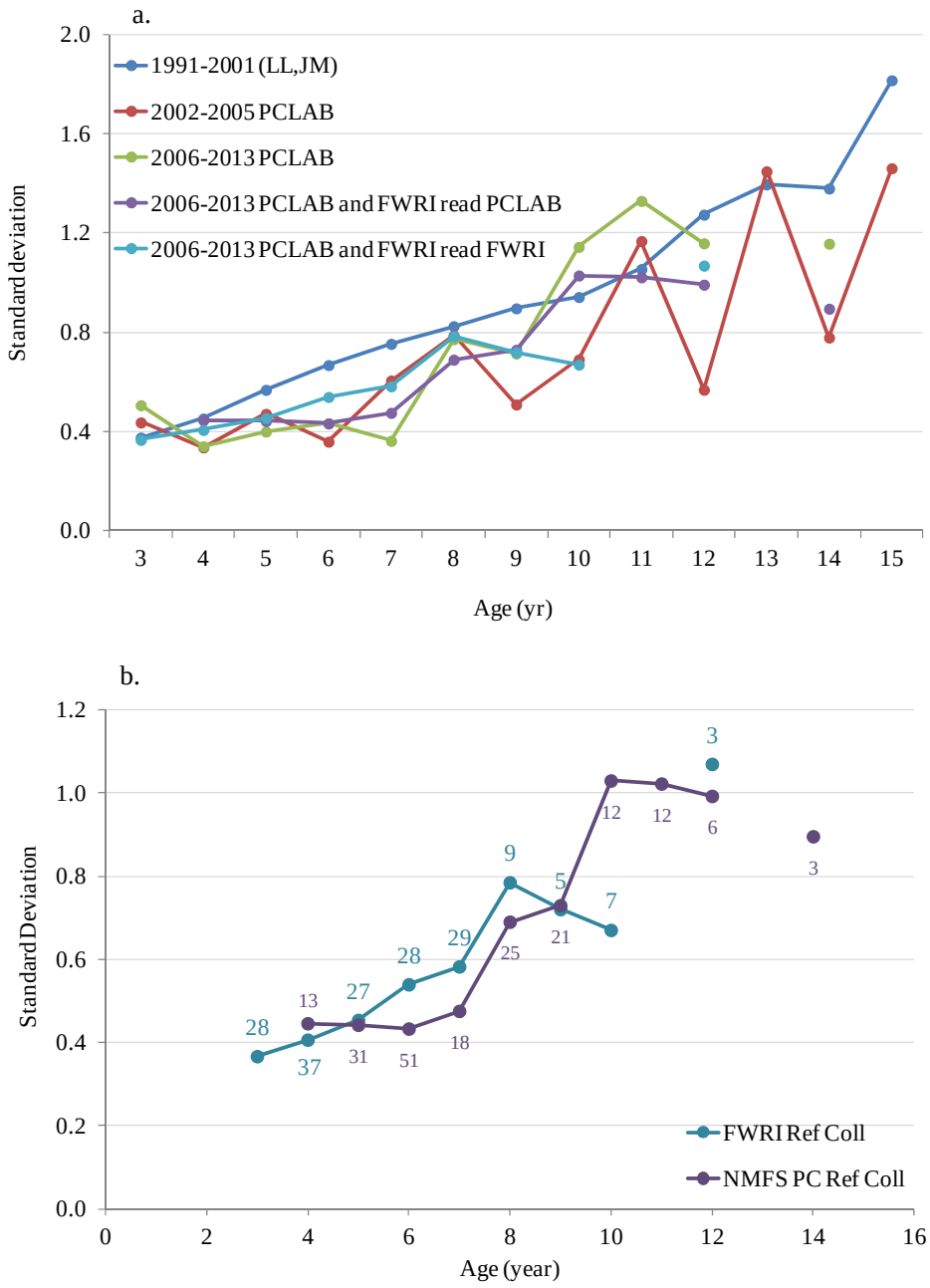


Figure 2. Standard deviation by age for red grouper reference collection readings by (a) particular time periods that reflect changes in primary reader at the PCLAB: 1991-2001 L. Lombardi and J. Mikulas historical reading, 2002-2005 PCLAB – PCLAB reference collection read by PCLAB, 2006-2013 PCLAB – PCLAB reference collection read by PCLAB; 2006-2013 PCLAB and FWRI read PCLAB - PCLAB reference collection read by PCLAB and FWRI, 2006-2013 PCLAB and FWRI read FWRI - FWRI reference collection read by PCLAB and FWRI and (b), overall average for all time periods and readers of PCLAB reference collection and overall average for reading of FWRI reference collection (sample size shown at data points in corresponding color per reference collection).