Utilizing Paired Readings and an Otolith Reference Collection to Determine Ageing Precision of Vermilion Snapper, (*Rhomboplites aurorubens)*

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Introduction

The National Marine Fisheries, Panama City Laboratory (PCLAB), Florida Fish and Wildlife Conservation Commission/Fish and Wildlife Research Institute (FWRI), and Gulf States Marine Fisheries Commission (GSMFC) including Alabama, Louisiana, and Texas provided age data for the SEDAR 45 vermilion snapper assessment. These data undergo extensive quality control standards, as the data is vital in understanding the health of fish stocks and the need for effective fisheries management. Standardizing ageing procedures and estimating reader precision of a given species commonly occurs using a reference collection. The current vermilion snapper reference collection (n= 200) is a compilation of sectioned otoliths from PCLAB, FWRI, and GSMFC to monitor reader precision within and among laboratories. Prior to the development of the reference collection, paired readings, where two readers aged a subset of fish, served as a means to determine reader precision.

There are three important objectives to this study: (1): to gauge PCLAB in-house reader precision during periods where changes in readers occur, (2) to compare reader precision among laboratories using a reference set, and (3) to report variability among ageing laboratories. Studies show a reference collection to be a useful tool in estimating the repeatability (i.e. precision) among readers (Campana, 2001: Allman, 2005).

Methods

Training readers

Vermilion snapper annulus patterns in thin sectioned sagittal otoliths are reported as difficult and require specialized training. Each ageing facility trains otolith readers specific to species by using a subset of sectioned otoliths assigned a consensus age such as reference collections**.** In addition**,** GSMFC holds an annual otolith processor meeting as a means to standardize ageing methods among Gulf of Mexico (GOM) ageing labs. The results of annual reference collection exchanges are discussed and any large ageing discrepancies are attempted to be resolved through group ageing exercises. Since 2013, the vermilion snapper reference collection has been circulated annually among laboratories contributing age data, including: PCLAB, FWRI, and GSMFC. For years prior to 2013, no data is available supporting the regular use of a reference collection. In 1999, 2005, and 2007, paired readings of a subsample of ages were used to estimate precision. R. Allman (PCLAB) has been ageing vermilion snapper since

1994, and is designated as primary reader for all years. Other main PCLAB contributors, during earlier years include B. Fable and J. Keese. The newest readers, C. Palmer and L. Thornton (PCLAB) began ageing in early 2015, having trained on the current reference set prior to assigning any ages.

The vermilion snapper reference collection is composed of 200 thin-sectioned sagittal otoliths prepared on slides (see Allman, 2007 for processing and ageing protocol). The age range, for the reference collection is 0-14 years, with fork lengths from 92 to 465 mm. All levels of otolith of readability are represented (i.e. good, readable, and difficult) which is necessary for an accurate estimate of precision as reported by Campana (2001). PCLAB contributed 50% of the sectioned samples all from 2009. FWRI contributed 13% of the sectioned samples to the reference collection and were a significant age contributor. Gulf States, including Alabama, Louisiana, and Texas, contributed 37% sectioned samples to the reference set. However, Texas is the only state used in reader precision determination, due to the large age contribution made to the overall sample set used for the SEDAR 45 (Table 1). Specific data for individual readers from FWRI and GSMFC were not available for inclusion in this report.

The quarter year distribution of samples in the reference collection as well as the SEDAR 45 data show relatively even distribution of samples throughout the year (Figure 1a). The ages and lengths usually observed in vermilion snapper sample distributions are represented within the reference collection and paired otolith samples; these values are similar to the final age data for SEDAR 45 (Figure 1b & 1c). The maximum reported age of vermilion snapper for GOM ages is 29 years (Table 4), as determined by a small selection of older fish that PCLAB readers re-aged.

Otolith Interpretation

Thin sections are read using a stereo microscope with magnifications ranging from 15-75x. Opaque, concentric bands on the dorsal side of the sulcus are counted as annuli. Preliminary research on band increment measurements from PCLAB determined the mean distance from the core to the first annulus is approximately 0.5 mm. Readers recorded the number of annuli as well as the edge type observed. Vermilion snapper are generally reported as difficult to age compared to other reef fish, due to difficulty identifying the first annulus and the diffuse nature of increments. To eliminate reader bias, no meristic data is available to readers other than the date of collection to insure proper age advancement when applicable.

Band count, edge type, and capture date are used to calculate final age (Jerald, 1983). There are six different classifications for edge types used among all ageing facilities. PCLAB uses edge type classifications 2, 4, and 6 (Table 2a). Edge type 2 indicates an opaque band on the edge of the otolith section, where band count and final age would be equal. Edge 4 indicates significantly narrow translucence, where band count and final age would be equal. Edge type 6 indicates a large translucence margin; if the sample capture date fell within January 1st through June 30th, then edge types of 6 were advanced one year. FWRI and GSMFC use edge type classifications of 1, 2, 3 and 4 (Table 2b). Edge type 1 indicates complete opaque banding on the edge of the otolith section. Edge type 2 indicates a narrow translucence. Edge type 3 indicates translucence observed as one third to two-thirds complete on the edge of the otolith section. Edge type 4 indicates complete translucence. With this classification series, if the sample capture date fell within January 1st through June 30th, then edge types of 3 and 4 were advanced one year. For edge types 1 and 2, age equaled increment count (Vanderkooy and Guindon-Tisdel 2003).

<u>Precision</u>

Three indices of precision are used to determine the overall accuracy among readers from all ageing labs. Average percent error (APE), coefficient of variation (CV), and precision (D) were calculated (Kimura and Anderl; 2005: Campana, 2001) from each lab for the reference collection; these indices were also calculated for PCLAB and the three earlier (1999, 2005, and 2007) sets of paired readings. Due to the lack of a regular reference collection prior to 2013, paired readings were used to determine reader variability. Ageing error was then estimated by calculating standard error among readers for each age.

In order to better approximate ageing error in older fish (>14 years old) PCLAB readers also re-aged a small subset (n=9) of known older age samples (Table 4). These older age groups comprise less than 1% of the total aged samples submitted for SEDAR45; this subset did estimate a 29 year longevity estimate for vermilion snapper, but specific ageing error and reader precision calculations were not completed for this small subset.

Results and Discussion

In all reading comparisons, standard deviation of average age is higher for vermilion snapper in comparison to other Lutjanids (Figure 2a). A trend of decreasing precision with increasing ages can also be seen for all reading comparisons. This trend may be a result of finer, diffuse bands in older fish. Low reader precision was noted for PCLAB paired readings conducted in 1999 as well as the PCLAB combined reading of the reference set (Table 3). A low sample size as well as lack of established ageing techniques in interpreting annuli may have resulted in low precision estimates reported for the 1999 readings. The overall deviation trend (Figure 2b) clearly increases with increasing age, with peaks at age 7, 8, 9, 10 and 12. Small sample sizes of older fish represented in the PCLAB paired readings as well as the reference set may also contribute to the increasing trend. PCLAB paired readings as well as reference collection readings had few older fish (<4% of fish ages 10 years or older). With preliminary research on location of the first increment, coupled with recently improved sectioning techniques and overall section quality, updating the vermilion snapper reference set should become a priority for future ageing comparisons.

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Table 1. Multiple readers contributed vermilion snapper ages to SEDAR 45. Listed are the percentages of age contribution by reader and by year (1994 – 2014). NMFS PC – National Marine Fisheries Service, Panama City Laboratory; FWRI – Florida Fish and Wildlife Research Institute; GSMFC – Gulf States Marine Fisheries Commission, TX –Texas, LA – Louisiana, AL-Alabama.

Year	R. Allman NMFS-PCLAB	B. Fable NMFS-PCLAB	C. Palmer NMFS-PCLAB	J. Keese NMFS-PCLAB	L. Thornton NMFS-PCLAB	FWRI	GSMFC AL	GSMFC LA	GSMFC TX
1994	100%								
1995	100%								
1996	100%								
1997	100%								
1998	100%								
1999	36%	63%							
2000	100%								
2001	100%								
2002	100%								
2003	1%					99%			
2004	100%								
2005	34%			6%		60%			
2006				98%		2%			
2007	43%			36%		2%	2%	3%	14%
2008	75%					6%	3%	3%	13%
2009				76%		12%	2%	2%	6%
2010				75%		15%	2%	1%	7%
2011	37%		44%			8%	3%		8%
2012	5%		15%		51%	13%	4%	1%	11%
2013	38%				29%	21%	4%	2%	6%
2014	29%		21%		24%	21%	4%	1%	

Table 2. Criteria for advancing vermilion snapper ages, (a) PCLAB and (b) FWRI and GSFMC

(a)

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

(b)

Collection Date	**Edge Type**	**Advance annulus count**
January 1 — June 30	1, 2	0
January 1 — June 30	3, 4	+1
July 1 — December 31	1, 2, 3, 4	0

Table 3. Overall reader indices of precision for NMFS Panama City Laboratory (PCLAB – R. Allman, B. Fable, J. Tunnell, J. Keese, C. Palmer, and L. Thornton), Florida Fish and Wildlife Conservation Commission /Fish and Wildlife Research Institute (FWRI), and Gulf State Marine Fisheries Commission (GSMFC)

Sample Years	Reader Pairing	n	APE	CV	D
1999	B. Fable and R. Allman Overlap Readings	167	17.89	13.86	6.93
2005	R. Allman and J. Tunnell Overlap Readings	356	6.82	5.00	2.50
2007	R. Allman and J. Keese Overlap Readings	529	5.64	2.30	1.15
2015	PCLAB overall readings of reference set	200	11.01	14.80	7.40
2015	PCLAB, FWRI, and GSMFC overall readings of reference set	200	13.69	3.23	1.61

Table 4. Subset of samples representing oldest vermilion snapper aged. R. Allman, L. Thornton, and C. Palmer (NMFS - PCLAB) re-aged these fish to help inform the model on the variability around natural mortality if natural mortality is based on longevity. Standard deviation (STD DEV) values among readers were calculated for this subset.

Capture Date	Fork Length	R. Allman Age	C. Palmer Age	L. Thornton Age	STD DEV
8/16/2001	396	29	26	28	1.53
4/23/2002	447	24	26	25	1.00
5/10/2004	415	27	27	28	0.58
3/18/2011	371	17	25	20	4.04
12/10/2012	360	24	25	26	1.00
6/21/2012	476	13	21	11	5.29
12/23/2013	418	22	23	24	1.00
5/21/2014	401	16	23	20	3.51
8/27/2014	339	16	24	17	4.36

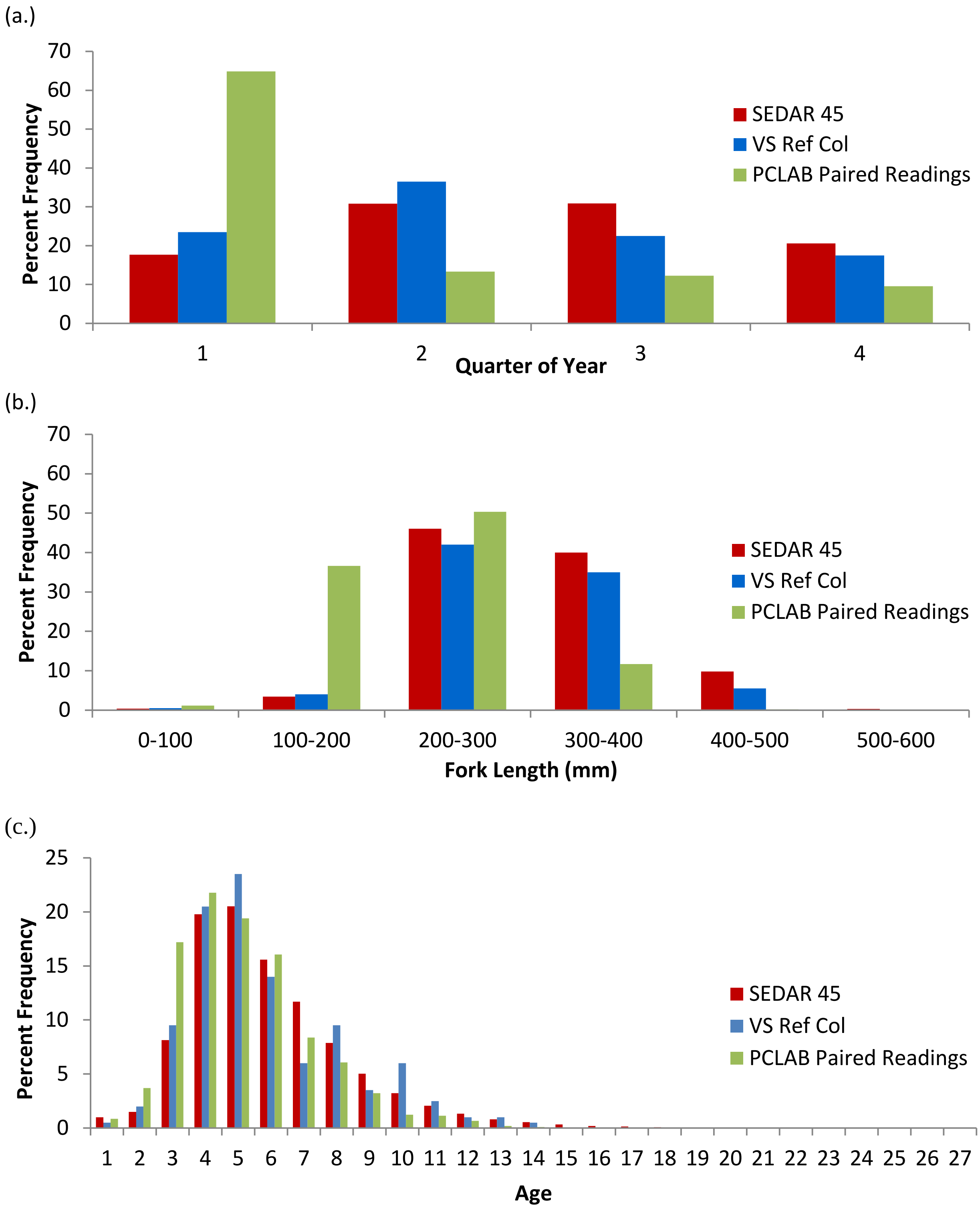
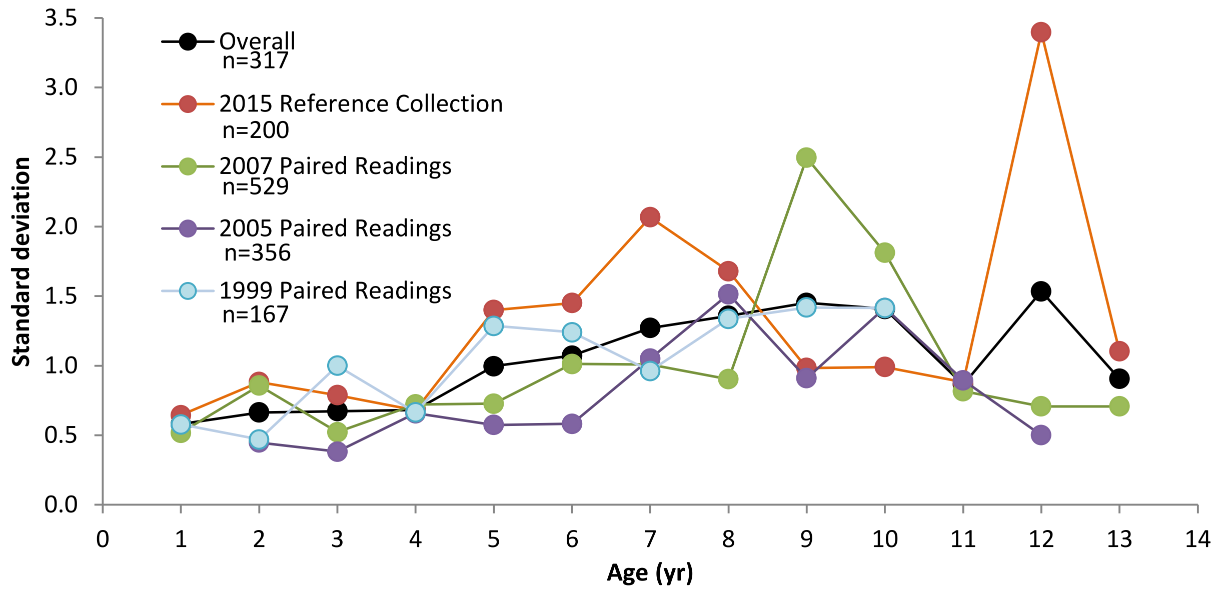


Figure 1. Description of the vermilion snapper ages by (a) quarter of year (b) length (c) age frequency for the vermilion snapper SEDAR 45 data (n=47,357), the PCLAB paired readings (n=1,052), and the reference set (n=200)

(a.)



(b.)

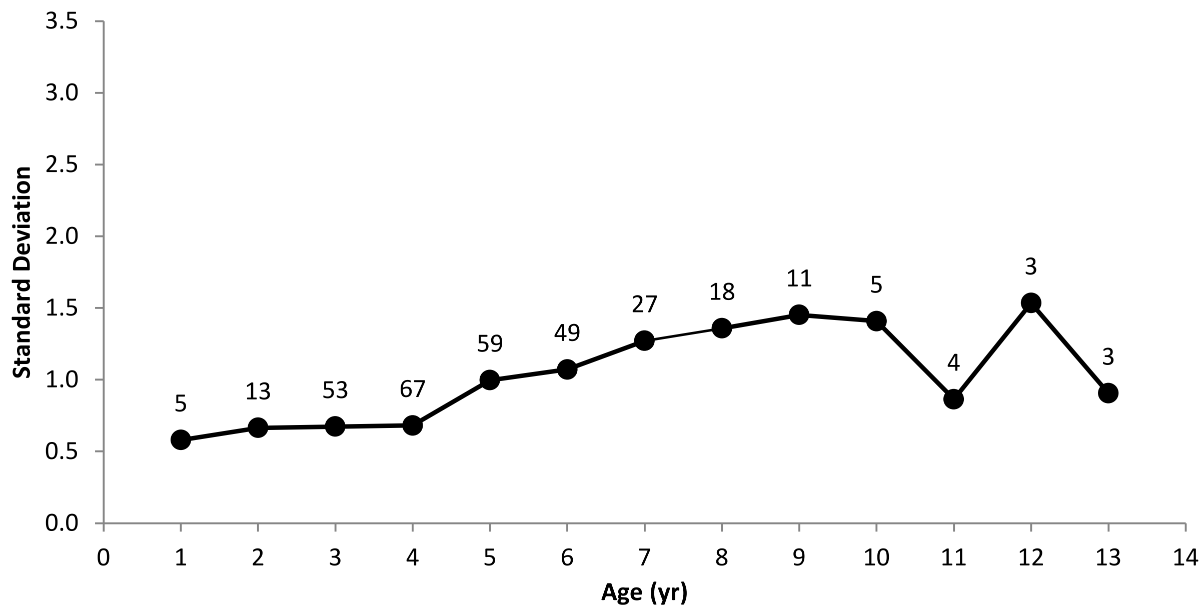


Figure 2. Standard deviation by age for vermilion snapper PCLAB age paired readings and reference collection readings by (a.) time periods that reflect changes in primary reader at PCLAB: 1999 B. Fable and R. Allman, 2005 R. Allman and J. Tunnell (FWRI), 2007 R. Allman and J. Keese, and 2015 reference collection readings, and (b.) overall ageing error for all time periods and ageing facilities.