

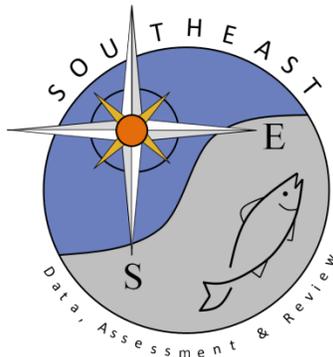
Apparent seasonal variations in age distributions and their linkage to
aging procedures used for red snappers collected from the Gulf of
Mexico

Ching-Ping Chih

SEDAR61-WP-18

16 November 2018

Updated: 6 December 2018



This information is distributed solely for the purpose of pre-dissemination peer review. It does not represent and should not be construed to represent any agency determination or policy.

Please cite this document as:

Chih, Ching-Ping. 2018. Apparent seasonal variations in age distributions and their linkage to aging procedures used for red snappers collected from the Gulf of Mexico. SEDAR61-WP-18. SEDAR, North Charleston, SC. 21 pp.

**Apparent seasonal variations in age distributions and their linkage to aging
procedures used for red snappers collected from the Gulf of Mexico**

CHING-PING CHIH

Submitted to the red snapper stock assessment team

on

February 6, 2018

Southeast Fisheries Science Center

National Marine Fisheries Service

National Oceanic and Atmospheric Administration

ABSTRACT

This report summarizes a controversial seasonal differences in age frequency distributions (AFDs) and age length keys (ALKs) in red snapper samples and presents evidence that these observed seasonal differences are related to the current age assignment procedures used for the SEDAR 52 red snapper stock assessment. The aging standards for the current age data sets are different for samples from different research laboratories. A significantly larger portion of age samples collected in the first 6 months were converted to the next age class for samples processed by the NMFS and GulfFIN laboratories as compared to those samples processed by academic research laboratories. The observed seasonal differences in AFDs/ALKs, which are biologically unlikely to occur, suggests that the proportions of samples being converted to the next age class in the current red snapper age data set are higher than what they should be. Problems in age class assignments not only influence the estimation of AFDs/ALKs but also affect the determination of growth parameters. These results suggest that the current age data used for the red snapper assessment may need revision. Possible short term and long term solutions to the aging problems are discussed.

INTRODUCTION

The current aging procedures for red snapper samples collected from the Gulf of Mexico (GOM) have been used since 2004 (SEDAR 7). As indicated in Allman et. al. (2004), the results of otolith edge analysis for red snappers determined by different research laboratories has varied (Fig 1). For red snappers, the opaque zone has been chosen to be counted as the annulus (Allman et.al. 2004). Two earlier academic studies (Wilson and Nieland, 2001; Patterson, et.al., 2001) showed that the percent of opaque otolith edges (opaque zone present on the edge of an otolith) for red snappers collected from GOM reached more than 90% during the first two months of the year, meaning that annual opaque edge formation had started during the first quarter (months 1-3) of the year for the majority of red snapper samples. In contrast, the results of Allman et al. showed that opaque edge formation for GOM red snappers did not peak until May. These differences in the peaks of the opaque edge formation periods make a big difference in the age assignment procedure. Based on the assumption that the peak of opaque edge formation was in May, current SEFSC aging procedures convert samples with otolith edges in the advanced translucent zone (see Methods and Fig 2) that were collected in season 1 (defined as months 1-6 in this report) to the next age class. As a result, a large proportion of samples collected in season 1 were converted to the next age class. In contrast, in the studies of Wilson, 2001 and Patterson, 2001, very small proportions of samples in the first quarter were converted to the next age class, and no samples in the second quarter were converted to the next age class since the annual opaque edge formation had been assumed to start during the previous winter (December) and the first quarter. For the current age data set (SEDAR 52), the proportions of samples converted to the next age class were also

much lower for samples processed by academic research laboratories (see below) as compared to those for samples processed by the NMFS and GulfFIN laboratories. The inconsistencies in age class assignment procedures can greatly influence the accuracy of estimated AFDs/ALKs and growth parameters.

One way to verify the assumptions used by current aging procedures is to compare the estimated AFDs/ALKs and length at ages (LAAs) from different seasons. Since the age conversion process was only applied to season 1 samples, any differences in AFDs/ALKs between the two season would suggest potential problems with the aging procedures. This study analyzed the seasonal differences in proportions at age (PAAs), ALKs, mean ages and LAAs for red snapper samples collected from commercial hand line fisheries from GOM. Hand line fishery samples were chosen because of the relatively larger sample sizes. Also, monthly distributions of different otolith stages were analyzed for samples from different laboratories and for samples of different ages.

The results from this study do not support the assumptions used by current SEFSC aging procedures and suggest that the current age dataset may need revision. Also, similar aging procedural issues are likely to exist for other SEDAR species. Short-term and long-term solutions to this problem are proposed.

MATERIALS AND METHODS

All age samples were from the red snapper age data sets provided for the SEDAR 52 red snapper stock assessment by the Panama City Laboratory, SEFSC. There were three major source of age samples: Panama City Laboratory (PC Lab samples; edge type codes PC-2,4,6), Gulf State Marine Fisheries Commission FIN database (GFIN samples; edge type codes 1,2,3,4) and academic research laboratories (e.g., Louisiana State University, University of South Florida, RS samples; edge type codes: RS-1,2,3,4,5,6). All PC Lab and GFIN samples used in this analysis were fisheries dependent samples, while a large portion of RS samples were fisheries independent samples.

The definition for different otolith stages (edge types) are different for different laboratories.

For GFIN samples (for graphical description, see Fig 2):

Edge types codes	Description
1	opaque zone present on edge
2	translucent zone <1/3 complete
3	translucent zone 1/3 to 2/3 complete
4	translucent zone 2/3 to fully complete

For PC Lab samples:

Edge type codes	Description
2_PC	opaque zone complete
4_PC	translucent zone forming to ½ complete
6_PC	translucent zone ½ to fully complete

For RS samples:

Edge type codes	Description
1_RS	opaque zone on edge to roughly 1/3 complete
2_RS	opaque zone on edge roughly 1/3 to 2/3 complete
3_RS	opaque zone on edge roughly 2/3 to entirely complete
4_RS	translucent zone initial forming to 1/3 complete
5_RS	translucent zone from 1/3 to 2/3 complete
6_RS	translucent zone from 2/3 to entirely complete

For comparisons of monthly distributions of otolith stage for samples from different laboratories, different edge types were grouped into three categories: OPZ stage - otolith edge in the opaque zone (codes: 1, 2_PC, 1_RS, 2_RS, 3_RS), NTZ stage - otolith edge not in the advanced translucent zone (codes: 2, 4_PC, 4_RS, 5_RS), and ATZ stage - otolith edge in the advanced translucent zone (codes: 3, 4, 6_PC, 6_RS). For the current SEFSC age assignment procedure, those samples collected in season 1 (months 1-6) and with ATZ otolith edge type were converted to the next age class. For example, a sample collected in June with an ATZ otolith edge type and an annuli count of 3 would be converted to age class 4.

Commercial hand line (CMHL) samples were used for the analyses of seasonal differences in AFDs /ALKs/mean ages/LAAs because of the relatively larger sample sizes. The term 'age' refers to age class in this report. All lengths are maximal total lengths in centimeters.

RESULTS

Distributions of different otolith stages

Monthly distributions of proportions of samples at different otolith stages were very different for RS samples compared to PC Lab and GFIN samples (Fig 3), particularly for the distributions of proportions at the OPZ stage. More than 90% of RS samples reached the OPZ stage in January. For both PC Lab and GFIN samples, the proportion of samples at the OPZ stage peaked in May with the highest level reaching only 40%. The proportion of samples at the ATZ stage for PC Lab and GFIN samples remained high for most of a year except for June to August. The large proportion of samples at the ATZ stage in season 1 led to a large percentage of samples being converted to the next age class.

Monthly distributions of otolith stages were also different for different age groups (Fig 4). For groups with an annuli count of 2, monthly distributions of proportions of samples at the OPZ stage were fairly even throughout the year with the highest level reaching only about 20% in May. Other age groups had more distinct peaks for the OPZ stage. Also, the proportion of samples at the ATZ stage were greater in the first quarter for older age groups compared to younger age groups.

Percentages of samples converted to the next age class (conversion rate) in season 1 were noticeably different for samples processed by different laboratories, especially in the first quarter (Table 1). Conversion rates for PC Lab and GFIN samples were much higher than those for RS samples in the first quarter. The conversion rates are also differed for different age groups (Table 2).

Seasonal differences in AFDs/ALKs

There were distinct seasonal differences in PAAs for commercial hand line samples (Figs 5 & 6). Note that season 1 PAAs and mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated PAAs. The reweighting process ruled out sampling factors in any observed seasonal differences in PAAs or mean ages. PAA 2 and PAA 3 estimated from samples collected in season 2 were consistently greater than those estimated from samples collected in season 1 for the years 2001-2016 (Fig 5). These results are biologically unlikely since the population for a particular age class can only decrease, not increase, as time goes by. A plausible cause for this result is that the conversion rate for season 1 samples was greater than it should have been. Because the age distributions in the younger ages were ascending with age class (i.e., $PAA3 > PAA2 > PAA1$, see Fig 7), the net result for an incorrectly higher conversion rate was a greater PAA in season 2 compared to season 1 (more samples were converted to the next age class than samples converted from the previous age class in season 1). Note that the mean lengths for age 2 in both seasons 1 and 2 were above the size limit. (The size limit for red snappers in the Gulf of Mexico (GOM) was 15 inches (total length 38.1 cm) for 2000-2008, and 13 inches (33.02 cm) after 2/2008). For commercial hand line samples, the mean total length for age 1 was 36.2 cm (season 2), and the mean total length for age 2 was 38.71 cm (season 1) and 39.65 (season 2).

The PAA estimated from samples collected in season 2 for age classes older than 5 were consistently smaller than those estimated from samples collected in season 1 (Fig 6 (b)-(e)). If there were no significantly different seasonal differences in PAAs, the chance for such a result to occur is extremely small ($((1/2)^{16})^4$). A plausible reason for these results is that the conversion rate for season 1 samples was greater than it should have been. Because the age distributions in the older ages were descending with age class (i.e., $PAA5 > PAA6 > PAA7 > PAA8$, see Fig 7), the net result for an incorrectly higher conversion rate was a smaller PAA in season 2

than in season 1 for age classes 5-8 (more samples were converted from the previous age class than samples converted to the next age class in season 1). Note: A similar trend occurred for samples with ages greater than 8, but the PAAs in those samples were too small to be included here. The seasonal changes in the age 4 class were not as consistent as the other age classes, probably because the age 4 class can be in either an ascending or a descending phase of an age distribution in different years.

The end result of the effects described in Figs 5 & 6 (i.e., greater proportions of younger fish and smaller proportions of older fish in season 2) was a consistently greater mean age for samples collected in season 1 than that for samples collected in season 2 (Fig 6(f)). Note that season 1 mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated mean ages.

There were similar seasonal differences in PAAs at fixed length intervals (ALKs, see Fig 8). These results confirm that the observed seasonal differences in PAAs was not due to differences in size distributions among seasons.

Quarterly differences in LAAs

LAAs estimated from samples collected in quarter 2 were equal to or lower than LAAs estimated from samples collected in quarter 1 for both PC samples and GFIN samples (Fig 9 (a), (b)). These consistently lower than expected LAAs for quarter 2 samples are biologically unlikely to occur and suggest that the age for quarter 2 samples may be overestimated (or more overestimated than quarter 1 samples).

DISCUSSION

Definition of otolith stages

The monthly distributions of otolith stages for PC Lab and GFIN samples differ significantly from those for RS samples, and from the results of studies from academic research laboratories (e.g., Wilson, 2001; Patterson, 2001). These differences in monthly distributions of otolith stages may be due to different definitions of otolith stages for each laboratory. For RS samples, the OPZ stage starts when the opaque zone begins to form on edge and ends with the completion of the opaque zone (see Methods). For PC Lab samples, the OPZ stage is defined as 'completion of opaque zone on edge'. For GFIN samples, the OPZ stage is defined as 'opaque zone present on edge' (see Fig 2 for general code descriptions for the OPZ stage from the

GSMFC aging procedure handbook). The peak of the OPZ stage for RS samples occurred in the winter months (Dec. to Feb.), which agrees with the general concept that opaque edges form during slow growth periods and translucent edges form during fast growth periods. For the aging procedures used by the academic research labs, the beginning of the opaque zone formation on an edge is used as the basis for identifying an age class. Since more than 90% of their samples reach the OPZ stage in the first quarter (Fig 3(c); also see Wilson, 2001; Patterson, 2001), there was no need to convert any ATZ samples collected in the second quarter to the next age class. In contrast, the peak of the OPZ stage for PC Lab and GFIN samples occurred in May and reached a maximum level of only around 40% (Fig 3(a), Fig 3(b)). Because only a very small proportion of PC Lab and GFIN samples reach the OPZ stage in the first quarter, it was assumed by the current aging procedure that all ATZ samples collected from season 1 had not formed an annulus for the year and would reach the OPZ stage later in the year.

What was even more problematic was how various labs defined the ATZ stage. For PC Lab and GFIN samples, samples at the ATZ stage included samples ranging from those 'with larger than 1/3 completed translucent zone on edge' to those at a stage where the opaque zone just beginning to form (Fig 2, code=3,4). As a result, samples at the ATZ stage dominate most months (except June through August; see Fig 3) for the PC Lab and GFIN samples. The current aging procedures assume the peak of the OPZ stage is in May, so all season 1 samples at ATZ stage were converted to the next age class. However, if the peak (> 90%) of the OPZ stage is during the winter (Dec. to Feb.), as reported in Wilson, 2001, Patterson, 2001; also see Fig 3(c)), then it is possible that a significant portion of the ATZ samples in season 1 (particularly in quarter 2) that were processed by the PC Lab and GFIN had already formed their annuli for the year. That is, converting those season 1 ATZ samples to the next age class end up overestimating the age (+1) for these samples. The possibility of an overestimation of age for season 1 samples is supported by the observed seasonal differences in AFDs/ALKs and in mean ages, and the observed quarterly differences in LAAs for the commercial hand line samples (Figs 5-9).

Another interesting finding from these analyses is that the monthly distributions of otolith stages may be age dependent (Fig 4). Future research is needed to examine whether different age assignment rules should be used for different age groups to accommodate differences in rates of development for different otolith stages for different age groups.

Seasonal differences in AFDs, ALKs and mean age

The observed seasonal differences in PAAs (Figs 5 & 6) and mean ages (Fig 6 (f)) are exactly what one would expect if the conversion rates were too high. When the age distributions were ascending with age class (PAA1-PAA3), the PAA for season 2 was greater than season 1, which creates a situation that is biologically impossible. The reason for this is

that the population for a given age class can only decrease, not increase, with time. When the age distributions were descending with age class (PAA5>PAA6>PAA7>PAA8), the PAA for season 1 was greater than season 2, which created a situation that was statistically unlikely to occur. Note that the reweighting of season 1 PAAs and mean ages by season 2 length frequency distributions rules out the effect of differences in sampling on the observed seasonal differences in PAAs and mean ages. The seasonal differences in ALKs (Fig 8) also confirm that these differences in PAAs were not due to differences in size distributions. These results support the idea that the age of a significant portion of season 1 samples may have been overestimated due to the aging procedures used.

Quarterly differences in LAAs

Problems in age assignment can also be seen in altered age length relationships (i.e., age at length (ALKs) and lengths at age). Any overestimation in age would result in underestimation of LAAs. The consistently equal or lower LAAs in quarter 2 samples, as compared to LAAs for quarter 1 samples (Fig 9) suggest that age in quarter 2 samples may be overestimated, which is consistent with the aging procedure problems mentioned above. Although LAAs can be influenced by factors such as non-random sampling and size limit harvesting, the consistent lower LAAs in quarter 2 samples seen in red snapper hand line samples and samples from other species (Chih, in preparation) indicates that there may be an overall problem in the aging method being used currently.

Problems in age class assignments can also affect the determination of fractional ages. The formulas used for determining the fractional age are based on age classes and are different between NMFS and other research labs (e.g., Wilson et al., 2001; Patterson et al., 2001; Saari et al., 2013). Since lengths at age and fractional age are used to determine growth curves, any problems in LAA estimation can have significant impact on the outcomes of stock assessments.

Potential impacts

The observed seasonal differences in AFDs and ALKs, and the influences of the potentially problematic aging procedures on estimated LAAs, fractional ages and growth parameters are likely to have a significant impact on the outcome of the current red snapper stock assessment. This aging procedure problem is likely to exist for other SEDAR species. Since most SEDAR assessments use age-structured assessment models, the observed aging problem can have a significant impact on all SEDAR assessments.

The observed effects of the aging procedures on estimated AFDs/ALKs depend on many factors, such as the actual annual peaks of OPZ stages, the shape of AFDs (i.e., the consistency of ascending/descending patterns over years), sampling intensity for different quarters/seasons/years, and variabilities in AFDs/ALKs/LAAs. As a result, the effects of aging

procedures on the estimated AFDs/ALKs/LAAs of other SEDAR species may not be as readily identifiable as they were for the red snapper commercial hand line samples.

Possible solutions

One possible short-term solution for the age determination problem is to use only samples collected from season 2 for estimating ALKs and growth parameters. By doing this, potentially biased samples from season 1 can be eliminated. Longer term solutions would include redefining the different otolith stages. A more detailed coding system such as the one used by Wilson (2001) and Patterson (2001) is needed. Such a coding system would provide more flexibility when different age assignment rules are adopted. Analyses of the effects of different aging rules on estimated AFDs/ALKs/mean ages/LAAs are also needed.

REFERENCES

Allman, R.J. Fitzhugh, G.R., Starzinger, K.J. and Farsky, R.A. 2004, Precision of age estimation in red snapper (*Lutjanus campechanus*), SEDAR7-DW-34.

VanderKooy, S. 2009, A practical handbook for determining the ages of Gulf of Mexico fishes', 2009, Gulf State Marine Fisheries Commission, publication No. 167.

Wilson, C.A. and D.V. Nieland, 2001, Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. Fish. Bull. 99:653-664.

Patterson, W.F. III, J.H. Cowan, Jr., C.A. Wilson and R.L. Shipp, 2001, Age and growth of red snapper, *Lutjanus campechanus*, from an artificial reef area off Alabama in the northern Gulf of Mexico. Fish. Bull. 99: 617-627.

Saari, C.R., J.H. Cowan, Jr. and K.M. Boswell, 2014, Regional differences in the age and growth of red snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico. Fish. Bull. 112: 261-273.

Fig 1 (a). Monthly distributions of proportions of samples at different otolith stages in the published literature (cited from Allman, et.al., 2004)

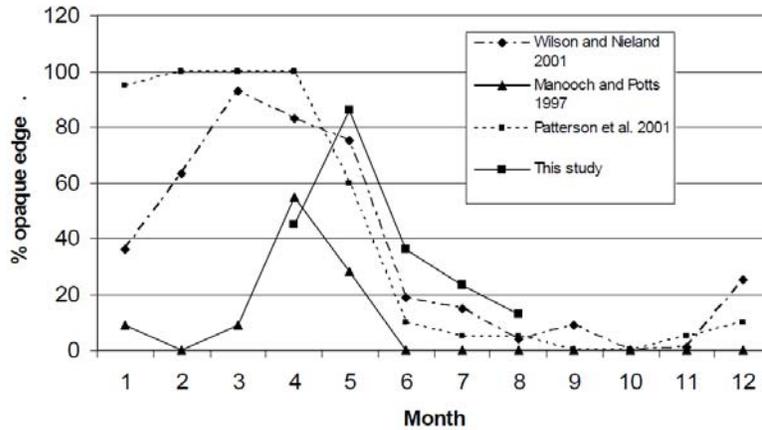


Figure 11. Results of red snapper otolith edge analysis from previous studies. Three studies measured the percent of opaque edges per month. The results from Wilson and Nieland (2001) and Manooch and Potts (1997) were interpolated from graphs similar to that shown. The results from Patterson et al. (2001) were interpolated from a bubble plot and two years were aggregated.

Fig 1(b). Monthly distributions of the proportion of red snapper age samples at different otolith stages (cited from Patterson, et al., 2001, Fish. Bull. 99:617-627). Total sample size = 862.

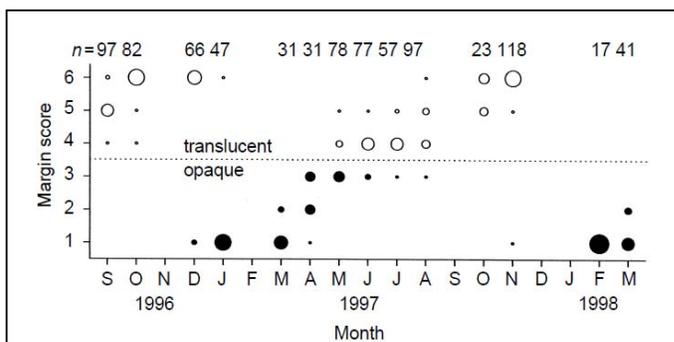


Figure 4

Plot of monthly margin edge scores of red snapper sagittae. Symbol size is relative to the percentage of fish with the corresponding margin edge score. Monthly sample size is given.

Fig 2. Graphical definition of different otolith stages from 'A practical handbook for determining the ages of Gulf of Mexico fishes', 2009, Gulf State Marine Fisheries Commission (GulfFIN), publication No. 167, page 48. (see Methods for the definitions of different codes).

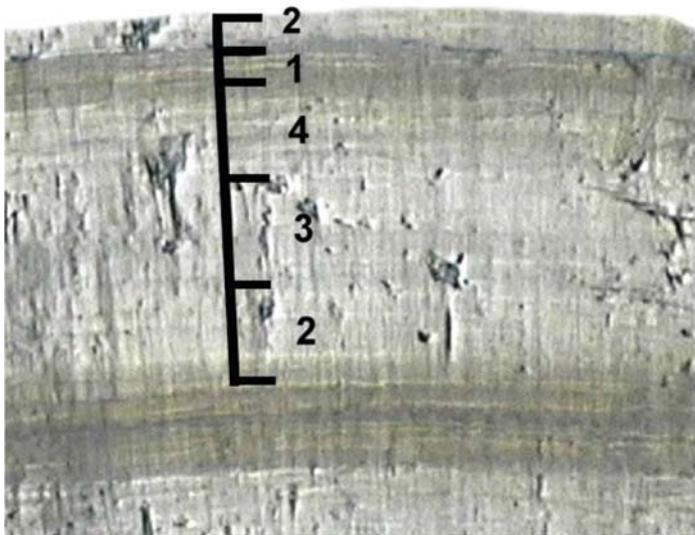
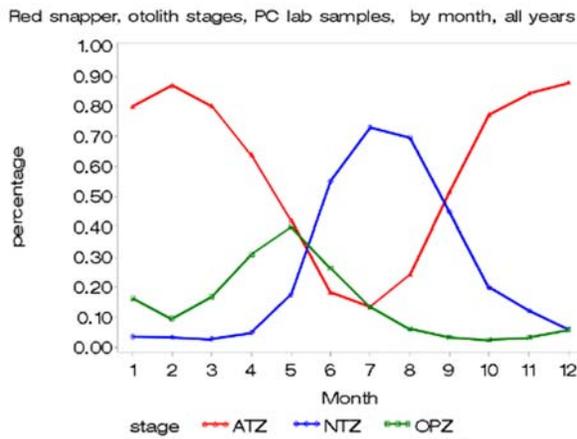
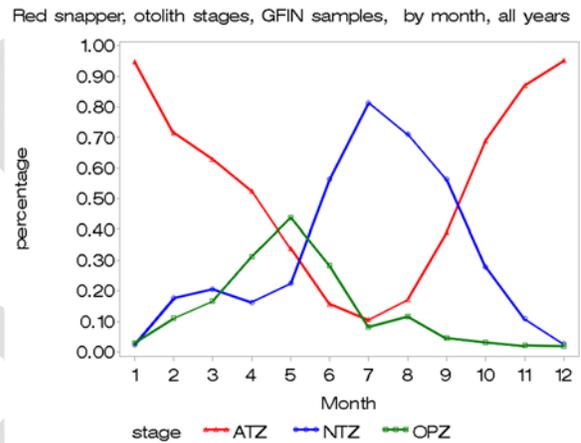


Fig 3. Monthly distributions of proportions of samples at different otolith stages in collected red snapper samples (see Methods for the definitions of different stages).

(a) PC Lab samples



(b) GFIN samples



(c) RS samples

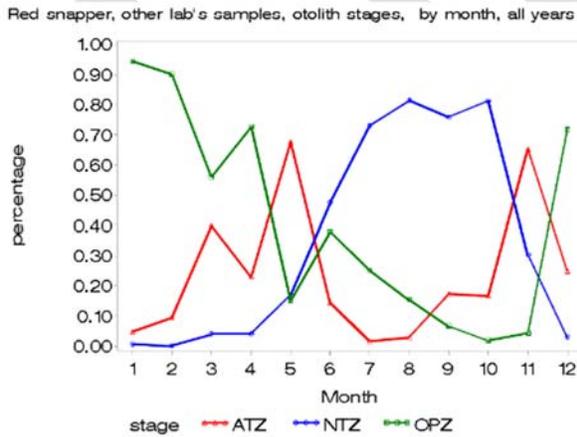
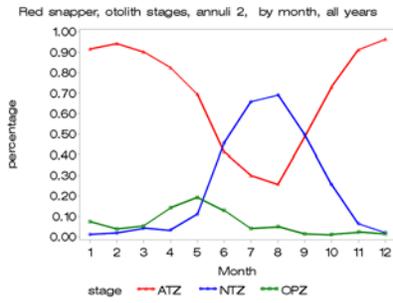
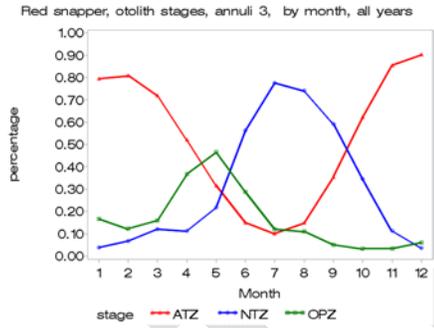


Fig 4. Monthly distributions of proportions of age samples at various otolith stages for different annuli groups. For the definitions of otolith stages, see Methods. All fisheries dependent samples are included in this analysis.

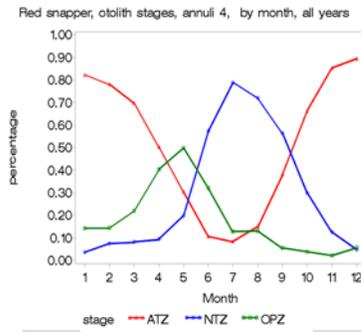
(a) Annuli=2



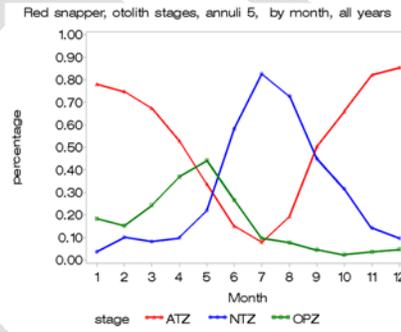
(b) Annuli=3



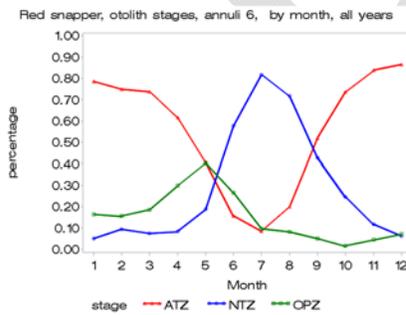
(c) Annuli=4



(d) Annuli=5



(e) Annuli=6



(f) Annuli=7

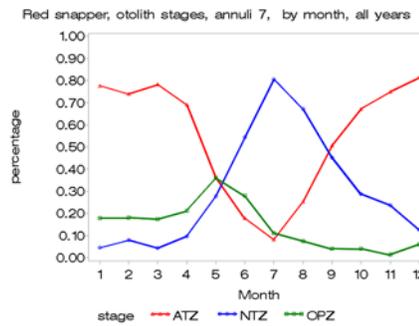


Table 1. Percentage of age samples converted to the next age class from different data sources in different quarters.

Labs	Quarter	n converted age	total n	Conversion rate
PC Lab	1	15592	18785	0.83
PC Lab	2	10632	27751	0.38
PC Lab	3	0	18361	0
PC Lab	4	0	13879	0
GFIN	1	5461	7681	0.71
GFIN	2	8438	32014	0.26
GFIN	3	0	23434	0
GFIN	4	0	10044	0
RS Lab	1	98	617	0.16
RS Lab	2	312	1132	0.28
RS Lab	3	0	4142	0
RS Lab	4	0	1713	0

Table 2. Percentage of age samples converted to the next age class from different data sources and different annuli groups in different quarters.

(1) All samples

Annuli	Quarter	n converted age	total n	Conversion rate
1	1	294	312	0.94
1	2	216	263	0.82
2	1	4035	4379	0.92
2	2	3948	6001	0.66
3	1	6357	8274	0.77
3	2	4909	16656	0.29
4	1	4415	5883	0.75
4	2	3377	14071	0.24
5	1	2485	3447	0.72
5	2	2579	9791	0.26
6	1	1506	2005	0.75
6	2	1623	5966	0.27
7	1	893	1167	0.77
7	2	983	3380	0.29
8	1	524	700	0.75
8	2	654	1948	0.34

(2) RS samples

Annuli	Quarter	n converted age	total n	Conversion rate
2	1	44	71	0.62
2	2	203	253	0.80
3	1	20	257	0.08
3	2	45	454	0.10
4	1	11	82	0.13
4	2	9	121	0.07
5	1	7	73	0.10
5	2	14	86	0.16
6	1	9	46	0.20
6	2	8	92	0.09
7	1	3	49	0.06
7	2	15	67	0.22
8	1	0	14	0
8	2	5	38	0.13

Fig 5. Seasonal differences in proportion at age (PAA) 2 and PAA 3 for commercial hand line (CMHL) samples. Note that season 1 PAAs were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated PAAs.

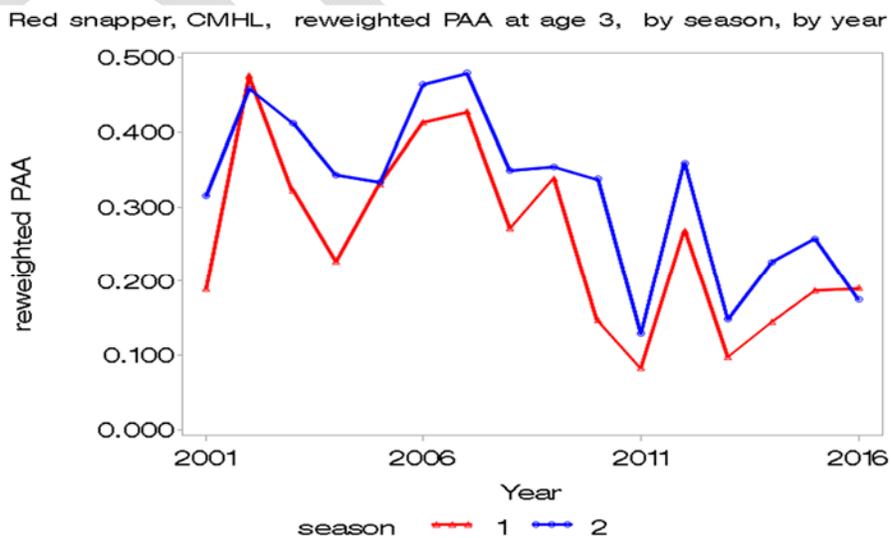
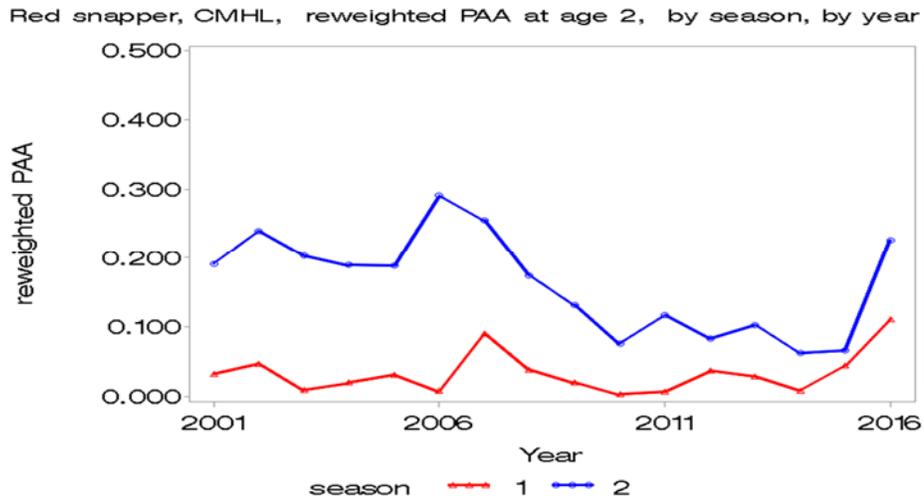
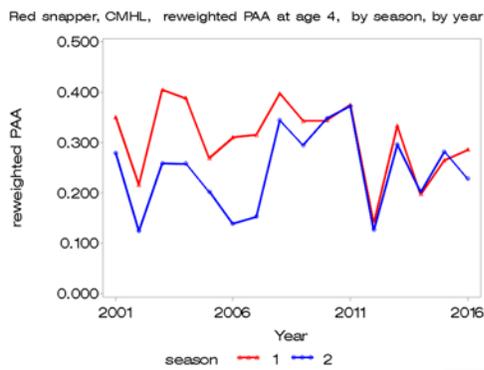
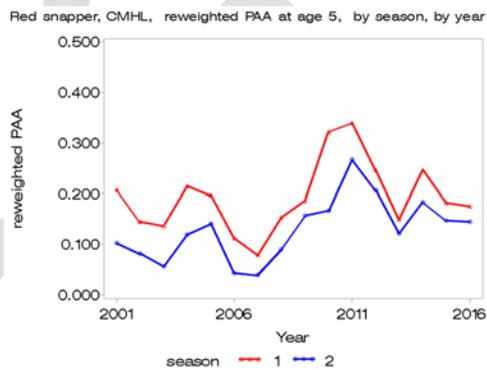


Fig 6. Seasonal differences in proportion at age (PAA) 4-8 for commercial hand line age samples (a-e). (f) Seasonal differences in mean age. Note that season 1 PAAs and mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated PAAs or mean ages.

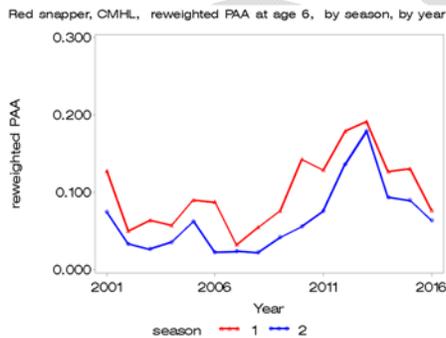
(a) PAA 4



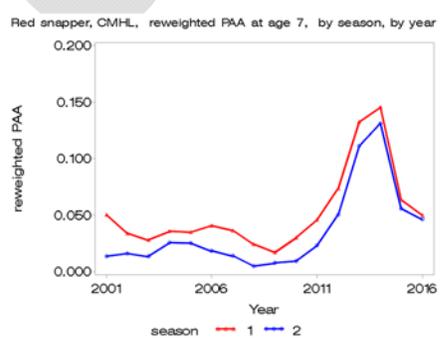
(b) PAA 5



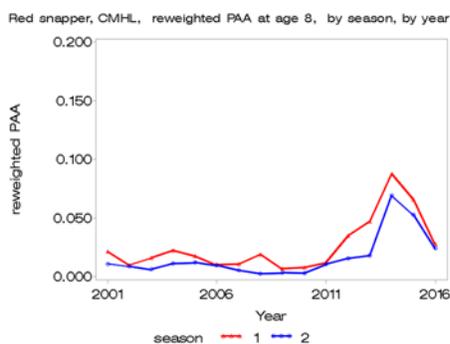
(c) PAA 6



(d) PAA 7



(e) PAA 8



(f) Mean age

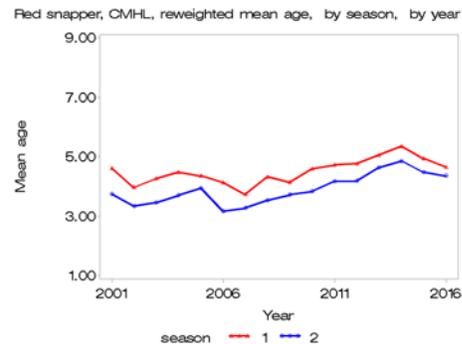


Fig 7. Seasonal differences in age frequency distributions (AFDs) for commercial hand line age samples.

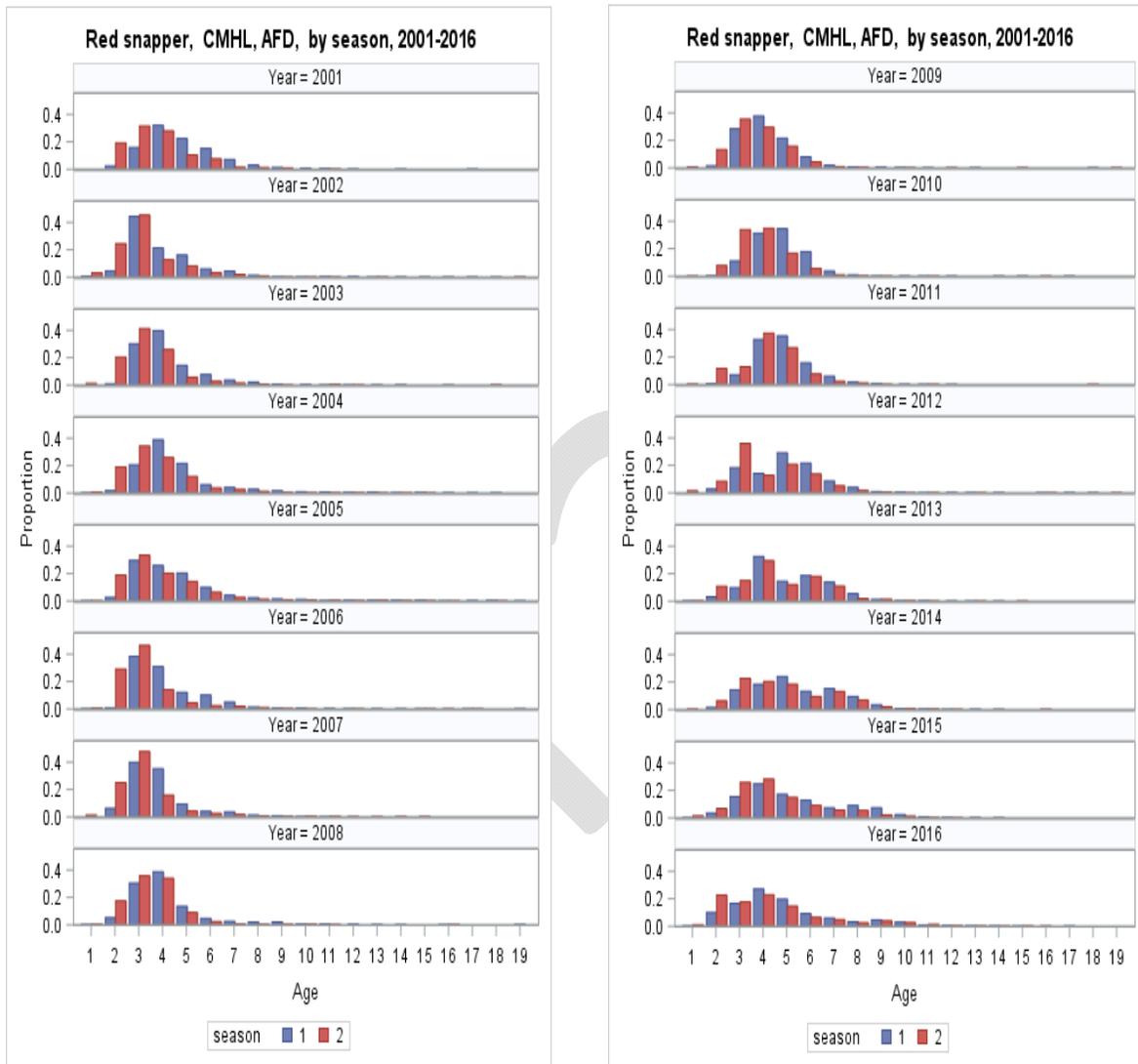
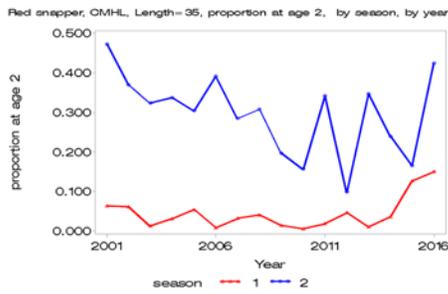
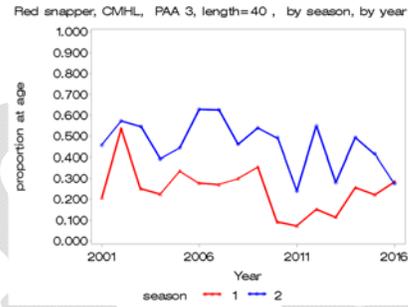


Fig 8. Seasonal changes in proportion at age (PAA) 2-7 at fixed maximal total length intervals of 5 cm (i.e. ALKs) for commercial hand line samples. (A length interval of 35 cm means '35cm<=length<40 cm')

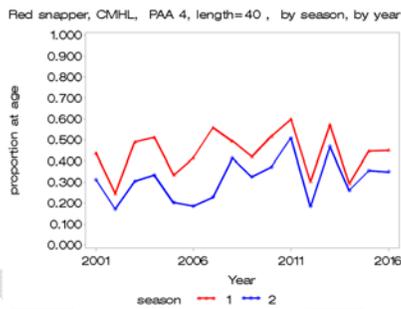
(a) PAA 2 at length 35 cm



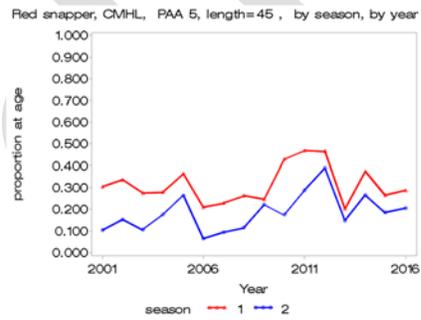
(b) PAA 3 at length 40 cm



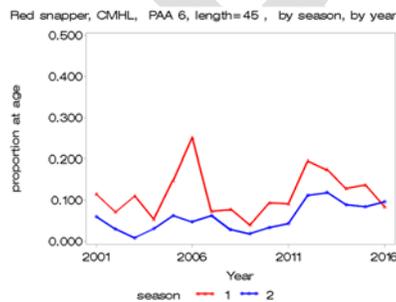
(c) PAA 4 at length 40 cm



(d) PAA 5 at length 45 cm



(e) PAA 6 at length 45 cm



(f) PAA 7 at length 50 cm

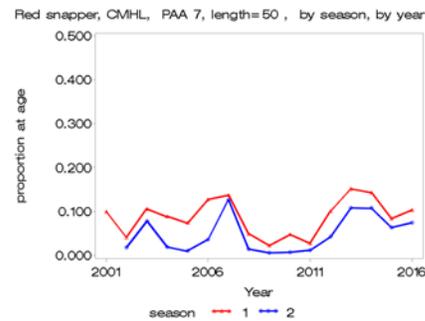
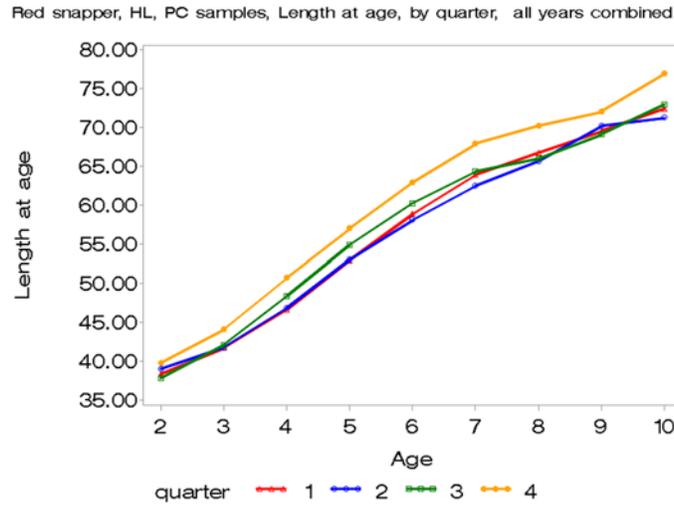


Fig 9. Quarterly changes in length at ages (LAAs) for red snapper commercial hand line samples processed by Panama City Lab (PC samples) and GulfFIN (GulfFIN samples).

(a) Panama City Lab samples



(b) GulfFIN samples

