On aging procedures for multiple reef fish species

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This report documents problems regarding current aging procedures used to age several SEDAR species. The three species investigated in this report include gray triggerfish, red groupers and gray snappers. In the case of gray triggerfish, the distribution of edge types and changes in lengths-at-ages between seasons suggest that the age data for this species may be biased and that an alternative assessment model (e.g., a length-based assessment model) should be considered. These analyses stress the importance of incorporating a validation step to the aging procedure so that similar types of errors may be avoided in the future.

This document is provided so that scientists involved with gray triggerfish, red grouper and gray snapper SEDAR assessments are informed about the pitfalls in current aging procedures, and so that necessary revisions or adjustments to SEDAR assessments based on a knowledge of these pitfalls may be made in a timely manner.

This study is part of a project that examines the aging procedures for multiple SEDAR species in the Gulf of Mexico. Documentation of aging procedure problems for red snappers (Chih, 2018) has been provided to the red snapper SEDAR analytic team during the latest red snapper SEDAR assessment.

Problems recognized in current aging procedures

A. Identification of age class

For age structured assessment models, age is defined by age class (i.e., fish born in a given calendar year belong to the same age class). Age class is determined based on the number of annuli and the edge type of an aging structure (e.g., otolith, dorsal spine, etc.). Aging structures for reef fish typically show repetitive annual rings that are composed of two zones – an opaque zone (OPZ, formed during slower growth periods) and a translucent zone (TZ, formed during faster growth periods). Although either an OPZ or TZ may be used to define an annulus, the OPZ has been chosen as the annulus for most SEDAR species. The edge type for an aging structure could be an OPZ or TZ, depending on the birth date and the time at which a fish is sampled. The monthly distributions for a particular edge type for a given fish population can be used to validate the use of that edge type as the annulus. For example, if the OPZ is used as the annulus, the monthly distribution for an OPZ edge type for a population should peak only once in a year. Also, the magnitude of the peak should be high enough to verify that a majority of the fish in a population form annuli each year.

Currently, aging results can differ greatly for samples processed by different labs because of differences in edge type definitions and in age assignment rules used by different labs.

1. Edge types definitions

Edge type definitions for the OPZ and TZ are different for samples processed by different government agencies and research labs. For example, definitions of OPZ edge types for red snappers include:

(a) Panama City Lab (PC Lab), SEFSC – the OPZ is complete on an edge.
(b) Gulf State Marine Fisheries Commission FIN database (GulfFIN)- the OPZ is present on an edge.
(c) Beaufort Lab, SEFSC – the OPZ begins to form on an edge (personal communication, Jennifer Potts, 2018).
(d) Academic research labs – the OPZ begins to form on an edge (Patterson et al., 2001). Note that the interpretation of 'the OPZ begins to form on an edge' may differ between the Beaufort Lab and the academic research labs. The OPZ edge type is further divided into 3 stages for samples processed by academic research labs.

Correct identification of an age class requires that the OPZ edge type be defined such that all samples that have an OPZ on the otolith edge are included (i.e., from the beginning to the completion of OPZ). The definition of OPZ edge types currently used by the PC Lab does not include the beginning stage of the OPZ. In addition, the PC Lab's definition of one edge type ('6_PC') is ambiguous in that it could mean either the OPZ or TZ is on the edge of an otolith (for details, see Chih, 2018). Such definitions for edge types can create problems for age assignments (see below).

2. Annual peaks for edge type distributions

Different definitions for the OPZ edge type resulted in differences in the observed annual peaks for the OPZ edge type. Usually it takes 1-2 months for an individual fish to form and complete an OPZ edge, while it may take 5-6 months for a fish population to form and complete an OPZ edge (VanderKooy, 2009). If the OPZ edge is recognized only when the OPZ edge is complete or nearly completed, then the annual peak for the OPZ edge would be delayed and be smaller in magnitude. For red snapper otoliths processed by academic labs (e.g., Wilson and Nieland, 2001; Patterson et al.,2001), the OPZ edge type peaks during the winter (Dec. to Mar.) with the maximum peak reaching more than 90%. However, for otoliths processed by the PC lab and GulfFIN, the OPZ edge type peaks in May with the maximum peak reaching only around 40% (Chih, 2018). The annual peak of an edge type is important because (1) it is used to validate the use of a particular edge type for an aging structure to define an annulus, and (2) it determines the age assignment rules.

3. Age assignment rules

For several SEDAR species, PC Lab and GulfFIN samples collected in season 1 (defined as months 1-6 in this report) and that had an 'advanced TZ edge type' were converted to the next age class. For red snappers, the definition for 'advanced TZ edge type' may range from one-third to one-half completion of the TZ stage to partial completion of the OPZ stage (for definitions of different edge types, see Chih, 2018). Such age assignment rules can overestimate age or samples collected in season 1, particularly in quarter 2, because of problems in defining edge types and identifying OPZ edge type peaks. As mentioned above, the peaks for OPZ edge types for PC Lab and GulfFIN samples may be delayed due to the definition of the OPZ edge type. The actual peak for the OPZ edge type for red snappers may be in the winter (Dec. to Mar.), as reported by Wilson and Nieland (2001) and Patterson et al. (2001). As a result, a significant portion of those season 1 PC Lab and GulfFIN samples (particularly quarter 2 samples) with 'advanced TZ edge type' may have already formed their annuli for a given year (i.e., the annuli for that particular year had already been counted). This means that the ages for many PC Lab and GulfFIN samples collected in season 1 may be overestimated by one year. The age assignment rule used by the academic labs only converted those samples with 'advanced TZ edge type' in the first quarter, not the second quarter, to the next age class. The definition for 'advanced TZ edge types' used by the academic labs was also different from what was used by the PC Lab and GulfFIN (for details, see Chih, 2018).

B. Verification of proper aging procedures

Since current age assignment rules for many species involve converting season 1 samples to the next age class, one way to verify age assignment rules is to check for any systematic seasonal variations in age frequency distributions or age-length relationships. If age is overestimated in season 1 samples, then systematic seasonal or quarterly differences in proportions-at-age (PAAs), mean ages, ages-at-length (age length keys (ALKs)) or lengths-at-age (LAAs) could exist.

1. Seasonal differences in PAAs, ALKs or mean ages

The problems with current aging procedures are demonstrated in the observed seasonal differences in PAAs, ALKs and mean ages in several species. As shown in the red snapper aging report (Chih, 2018), there are consistent and significant seasonal differences in PAAs, ALKs and mean ages, which indicate that age of samples collected from season 1 may have been overestimated. Results from red grouper and gray snapper show similar overestimations (see below). One caveat is that the observed effects of aging procedures on estimated PAAs/ALKs may not be easily identifiable for some species even when overestimation of age did occur. Factors that may obscure the effects of aging procedures include the inconsistencies of ascending/descending patterns of age frequency distributions over years, sampling intensities for different quarters/seasons/years, and variabilities in PAAs/ALKs (see Chih, 2018 for details).

2. Quarterly/seasonal differences in LAAs

   In general, quarterly LAAs for combined age classes should gradually increase (i.e. Q1-LAA < Q2-LAA < Q3-LAA < Q4-LAA). When aging assignment errors occur that affect age-length relationships, systematic deviations from a gradual increase would be observed. For example, Q1-LAAs for gray triggerfish samples are consistently higher than LAAs for other quarters for fish younger than 7 years old (see below). It should be noted that the variabilities for LAAs are typically higher for older fish, which can obscure the quarterly/seasonal increases in LAAs. Also, LAAs may be influenced by size limits and sampling irregularities, so the effect of aging procedures on LAAs may not be easily identifiable in some species.

Evidence of aging procedure problems for individual species

1. Gray triggerfish

   (a) Edge type distributions

   In gray triggerfish, the dorsal spine is used as an aging structure and TZ is used for counting annuli. The monthly distributions of TZ edge types for gray triggerfish dorsal spines processed by different labs differed noticeably (Fig 1 (a)). In particular, the TZ edge type distributions for samples processed by the PC Lab and GulfFIN do not show an annual peak. The monthly distributions of TZ edge types for gray triggerfish samples processed by the Beaufort Lab showed a distinct peak in April/May (Burton, 2015). These differences in edge type distributions led to different age assignment rules for these labs. For the PC lab and GulfFIN samples, no conversion to the next age class was done, while season 1 samples with an advanced OPZ edge type stage were converted to the next age class for samples processed by the Beaufort Lab (Burton, 2015). The lack of any peak in TZ edge type distributions for PC Lab and GulfFIN samples means that the TZ edge type, as currently defined by the two labs, may not be appropriate for identifying an age class (i.e., TZ, as currently defined by the two labs, may form more than one time in a year or not at all).

(b) Quarterly LAAs

The procedural problems with gray triggerfish dorsal spine samples processed by the PC Lab and GulfFIN can be seen in comparisons of quarterly LAAs for different ages. LAAs estimated for Q1 samples were consistently greater than LAAs estimated for other quarters for fish younger than 7 years old (Fig 1(b)). Similarly, season 1 LAAs were consistently greater than season 2 LAAs for fish younger than 7 years old. These results are biologically unlikely since fish in the same age class should not be smaller in the second season. These findings suggest that current aging procedures for gray triggerfish used by the PC Lab and GulfFIN may need revision.

2. Red grouper

(a) Edge type distributions

Otoliths are used to determine the ages of red groupers, and the OPZ is used to count annuli. As mentioned above, the OPZ edge type is defined as 'completion of OPZ stage' for PC Lab samples. Such a definition of the OPZ edge type could delay the timing of the peak for this edge type, leading to a low apparent maximum level of the annual peak. For red grouper, the peak of this OPZ edge type as defined by the PC Lab takes place in May and reaches a maximum of around 30-35%, only slightly above the 15-25% levels seen in other months (Fig 2(a)). This nearly flat distribution for the OPZ edge type does not support the assignment rule being used, which converts all season 1 samples with 'advanced TZ edge type' to the next age class. Since the 'advanced TZ edge type' includes samples that have one-third to one-half of the TZ completed, some season 1 'advanced TZ edge type' samples may already have annuli formed for the year. That is, the age for some Q1 and many Q2 samples may have been overestimated.

An additional issue for red grouper aging procedures is that, for samples collected in November and December, different aging rules were used. November and December red grouper samples with completed OPZ edge types were converted to the previous age class (representing about 11%, ranging from 0 to 66%, of red grouper age samples collected in November and December). Note that this particular conversion rule was not used for other SEDAR species (e.g., gag grouper, red snapper, gray snapper, etc.) in the Gulf of Mexico. Since the proportions of OPZ edge types were distributed relatively evenly throughout the year and only peaked slightly in May (similar to red snapper, gray snapper and gag grouper), the OPZ edge type for a given year class could have occurred in winter. For example, red grouper samples from November and December with the OPZ edge type may have had only one-third to one half of the TZ completed if they were caught in the second quarter (i.e., they would have been converted to the next age class by the currently-used assignment rule if they were caught in the second quarter). Thus, there is little to no evidence to support assigning red grouper November and December samples with an OPZ edge type to the previous age class.

(b) Seasonal differences in PAAs, ALKs and mean ages

Seasonal differences in PAAs, ALKs and mean ages for red grouper samples are not as distinct as those for red snappers (Chih, 2018) and gray snappers (see below). This may be due to a high variability in the age frequency distribution between years (i.e., the inconsistencies of ascending/descending patterns of age distributions between years) and the relatively small age sample sizes for red groupers. However, some notable seasonal differences for red grouper samples have occurred: (1) consistent seasonal differences in ALKs and PAAs for age classes 4 and 5 (Fig 2(b)) and (2) seasonal differences in mean ages found for both long line and hand line samples (Fig 2(b)). Note that season 1 PAAs and mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influence of differences in length distributions between the two seasons on the estimated PAAs and mean ages. The reweighting process ruled out sampling factors in any observed seasonal differences in PAAs or mean ages.

3. Gray snapper

(a) Edge type distributions

The definitions of otolith edge types for gray snapper are the same as those for red snapper. Similar to red snapper, the maximum level of the annual peak of the OPZ edge type reached only a relatively low level (about 20%) for PC Lab samples (Fig 3(a)). This relatively flat distribution for the OPZ edge type does not support the assignment rule being used, which converts all season 1 samples with 'advanced TZ edge type' to the next age class. A similar low peak was also observed for samples processed by the Beaufort Lab.

(b) Seasonal differences in PAAs, ALKs and mean ages

Similar to red snappers, significant and consistent seasonal differences in PAAs, ALKs and mean ages were seen in gray snapper samples collected in the Monroe County area (Figs 3(b) & 3(c)). Only Monroe County samples are included in these analyses because of the drastic differences in length/age frequency distributions between Monroe and non-Monroe County samples and the relatively small sample sizes for non-Monroe Counties samples. 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 and mean ages. These observations suggest that the age for season 1 gray snapper samples may also have been overestimated and need revision.

Conclusions

The analyses presented in this report and the red snapper aging report (Chih, 2018) suggest (1) that the edge type definitions used by the PC Lab and GulfFIN may have led to inaccurate age assignments for several SEDAR species, and (2) that the age assignment rules used by these labs created systematic seasonal differences in PAAs, ALKs, and mean ages, or quarterly differences in LAAs, that are not biologically likely. These age assignment problems could affect the estimation of both age frequency distributions and growth curves, and significantly influence the outcomes of stock assessments.

The findings presented in this report and the red snapper aging report suggest that the currently used definitions of edge types and age assignment rules may need revision for several species currently undergoing SEDAR assessments (red snappers, gray snappers, gray triggerfish and red groupers). In particular, for species where the OPZ is used to count annuli, the OPZ edge type should be defined in a way that would allow all samples that have an OPZ on the edge of the otolith (i.e., from the beginning of the formation of the OPZ to the completion of the OPZ stage) to be included. This would avoid the confusion when both the OPZ and TZ stages are included in the same edge type (e.g., '6_PC' for PC Lab samples). A more detailed coding system such as the one used by Patterson et al. (2001) would provide more flexibility when different age assignment rules are adopted. For ongoing SEDAR assessments, alternative methods (e.g., using second season ALKs or length based assessment models) may be needed to address the problems with age data.

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

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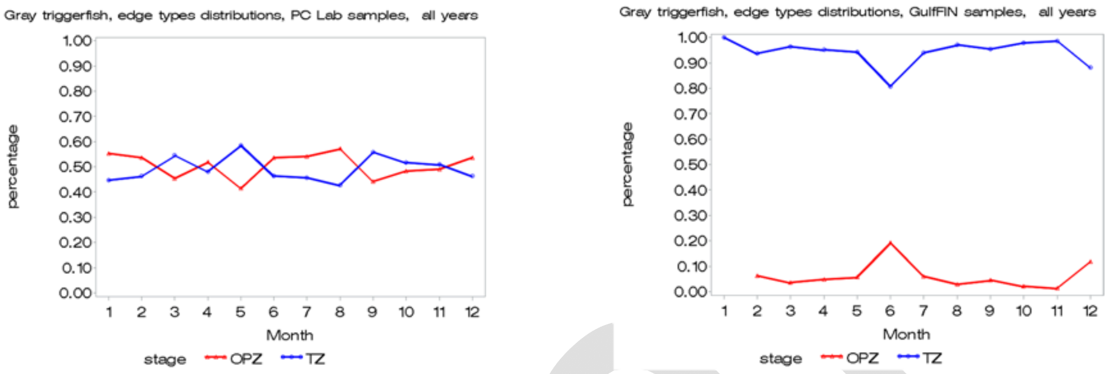
Fig 1 (a). Monthly distributions of edge types for gray triggerfish dorsal spine samples processed by different Labs (i) Panama City Lab samples, (ii) GulfFIN samples, and (iii) Beaufort Lab samples (From Fig 1 of Burton et al., 2015) (TZ -translucent zone, OPZ - opaque zone). Note that the distributions of the TZ edge type for Panama City Lab and GulfFIN samples do not satisfy the requirement for using TZ as the annulus.

(i)Panama City Lab samples (ii) GulfFIN samples



(iii) Beaufort Lab samples (TZ edge type)

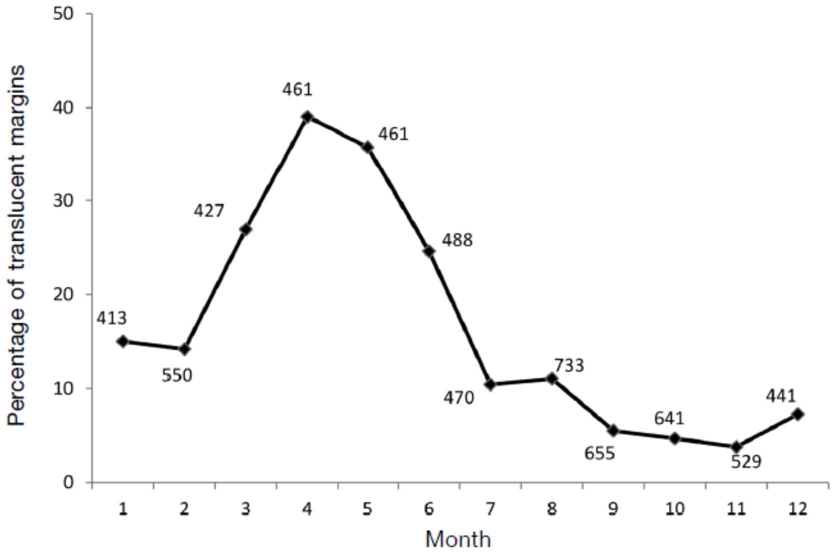
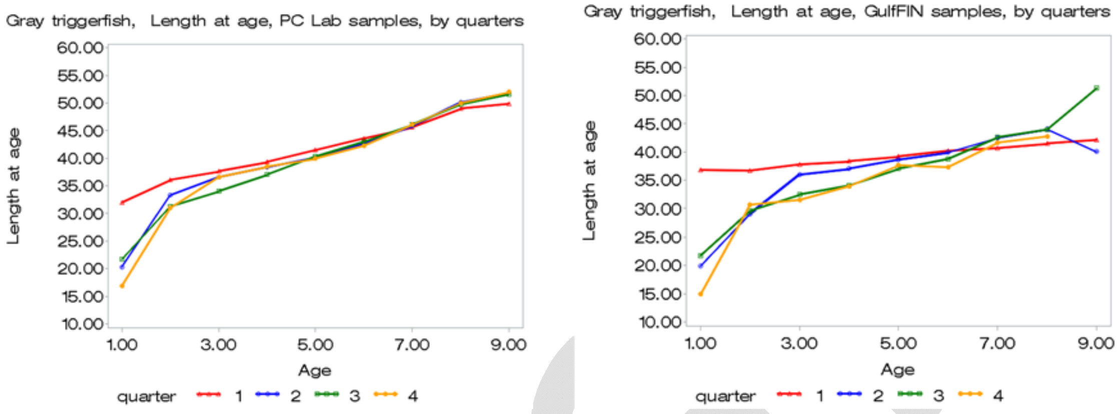


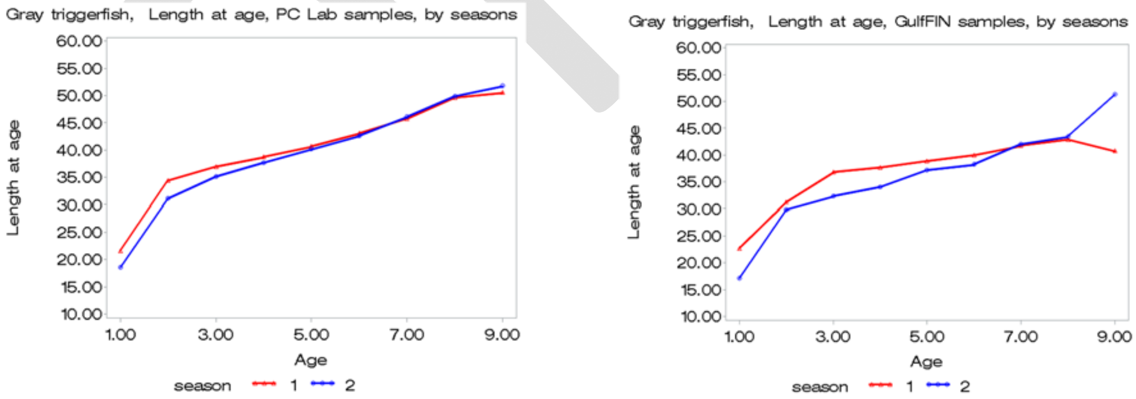
Fig 1(b). Comparisons of quarterly and seasonal lengths at age for different ages of gray triggerfish samples processed by different labs (season 1: months 1-6, season 2: months 7-12). Note that the values of LAAs do not follow the normally expected pattern that Q1-LAA < Q2-LAA < Q3-LAA < Q4-LAA.

(i) Quarterly lengths at ages


Gray triggerfish, Length at age, PC Lab samples, by quarters

Gray triggerfish, Length at age, GulfFIN samples, by quarters

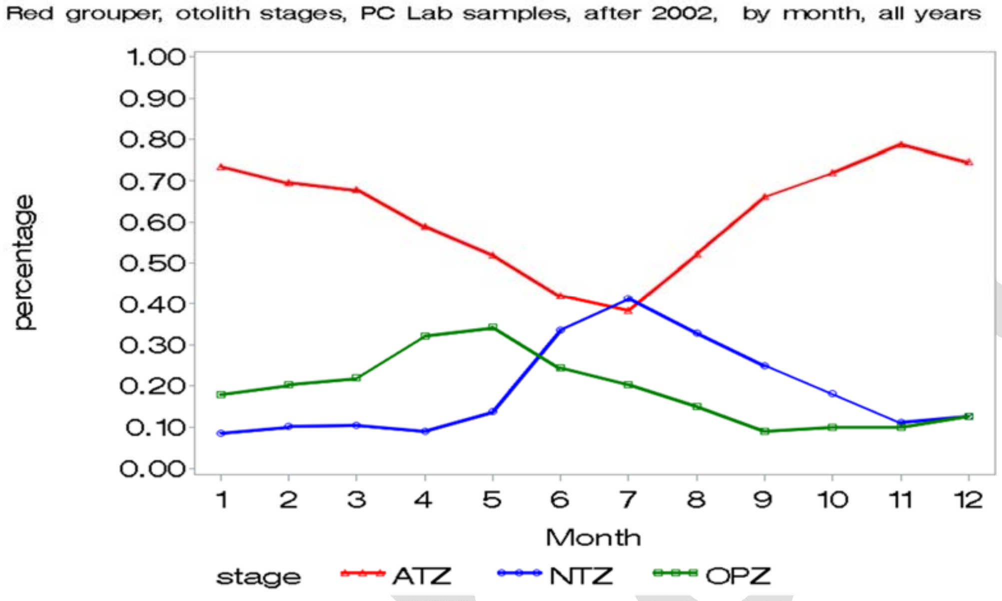
(ii) Seasonal lengths at ages


Gray triggerfish, Length at age, PC Lab samples, by seasons

Gray triggerfish, Length at age, GulfFIN samples, by seasons

Fig 2 (a). Monthly distributions of edge types for red grouper otolith samples processed by different Labs: (i) Panama City Lab and (ii) GulfFIN. OPZ- opaque zone, ATZ- advanced translucent zone, NTZ- non-advanced translucent zone. For detailed definition of edge types for different Labs, see Chih, 2018.

(i) Panama City Lab



(ii) GulfFIN

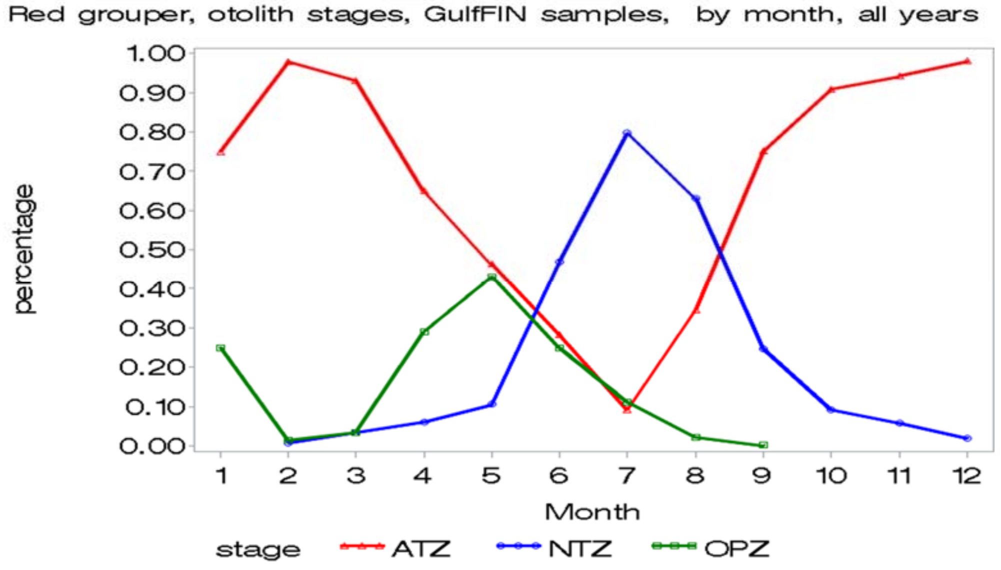
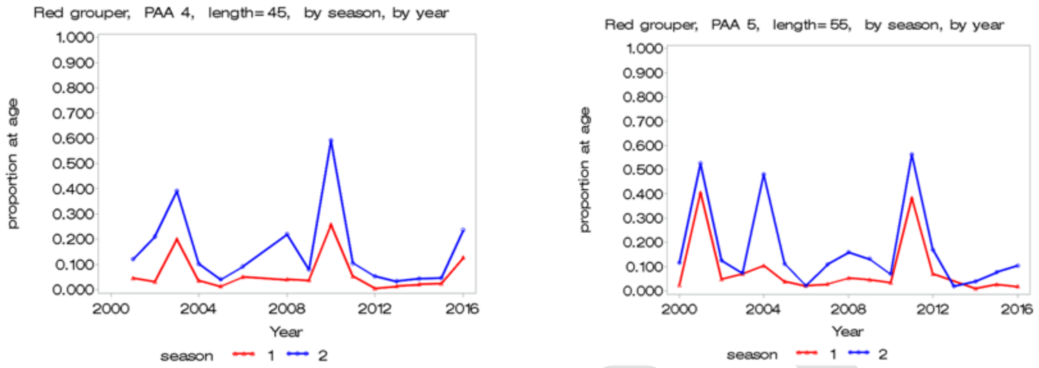


Fig 2 (b) Seasonal differences in proportion at ages (PAAs), age at lengths (ALKs) and mean ages for red grouper samples. Note that season 1 PAAs and mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated PAAs.

(i) Age at length



(ii) Reweighted proportion at ages (PAAs)

(iii) Reweighted mean ages

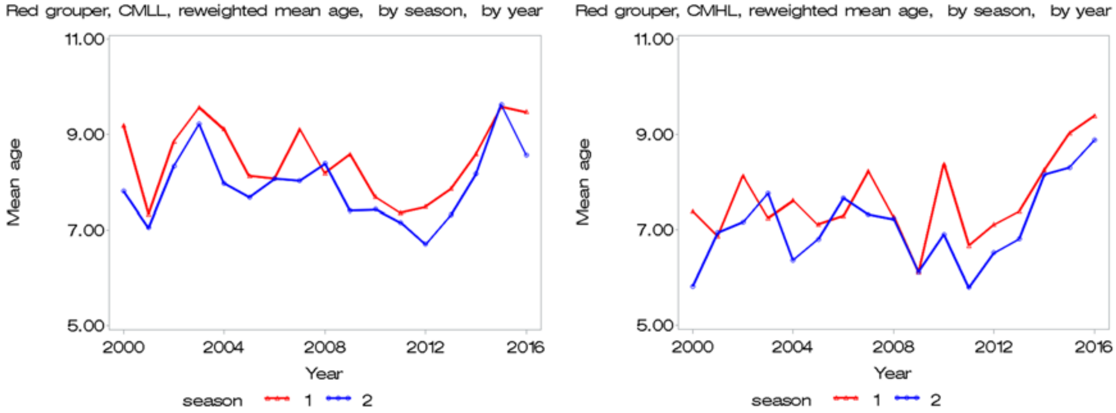


Fig 3 (a). Monthly distributions of edge types for gray snapper otolith samples processed by different Labs: (i) Panama City Lab, (ii) GulfFIN and (iii) Beaufort Lab. OPZ-opaque zone, ATZ-advanced translucent zone, NTZ-non-advanced translucent zone. For a detailed definition of edge types for the different Labs, see Chih, 2018. Note: Based on the original data document, the edge type definition for gray snapper samples processed by the Beaufort Lab was the same as that used by GulfFIN.

(i) Panama City Lab

(ii) GulfFIN

(iii) Beaufort Lab

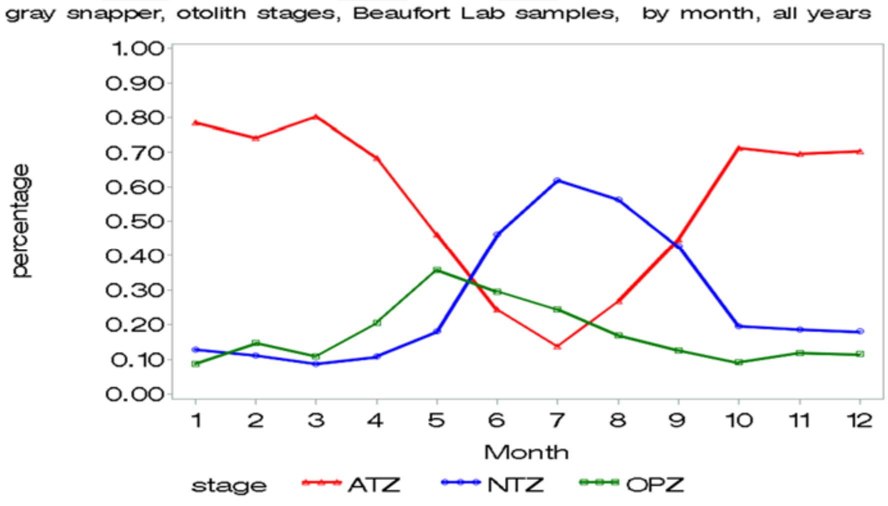
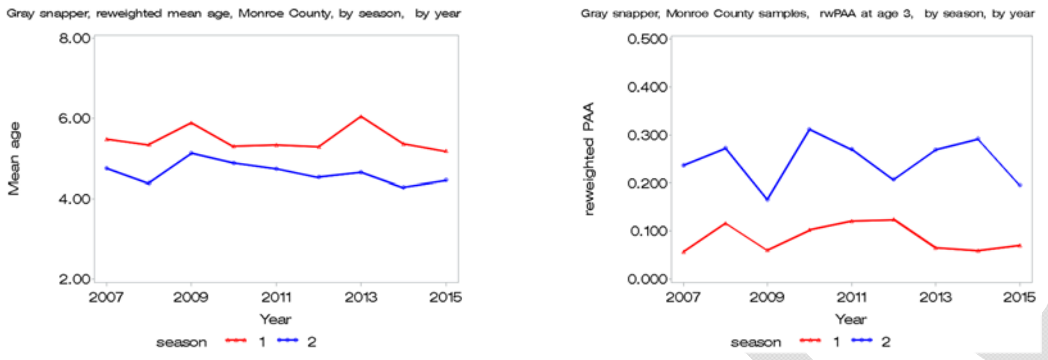


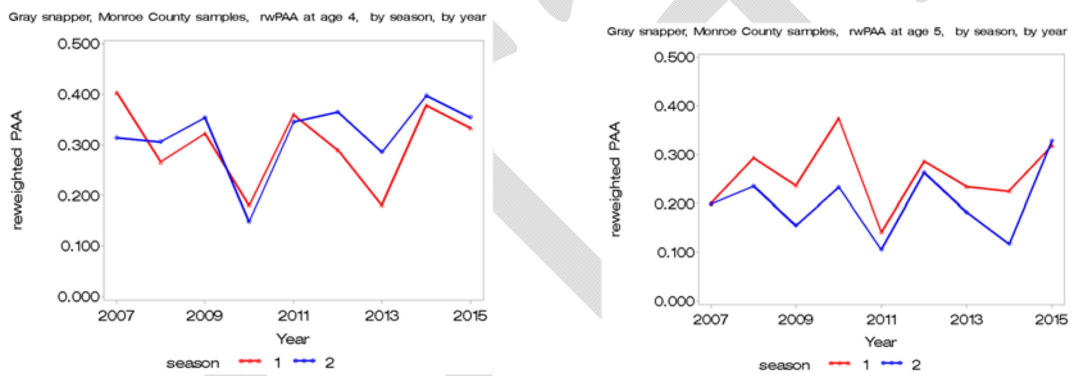
Fig 3 (b) Seasonal differences in mean ages and proportion at ages (PAAs) for gray snapper samples collected from Monroe County. Note that season 1 PAAs and mean ages were reweighted by the length frequency distributions of season 2 samples to eliminate the influences of differences in length distributions between the two seasons on the estimated mean ages and PAAs.


(i) Mean ages

(ii) reweighted PAA 3

(iii) reweighted PAA 4

(iv) reweighted PAA 5



(v)reweighted PAA 6

(vi) reweighted PAA 7

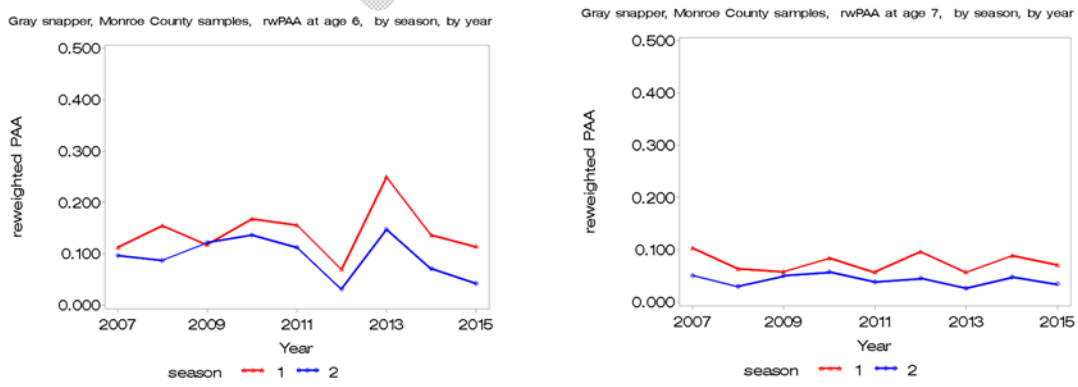
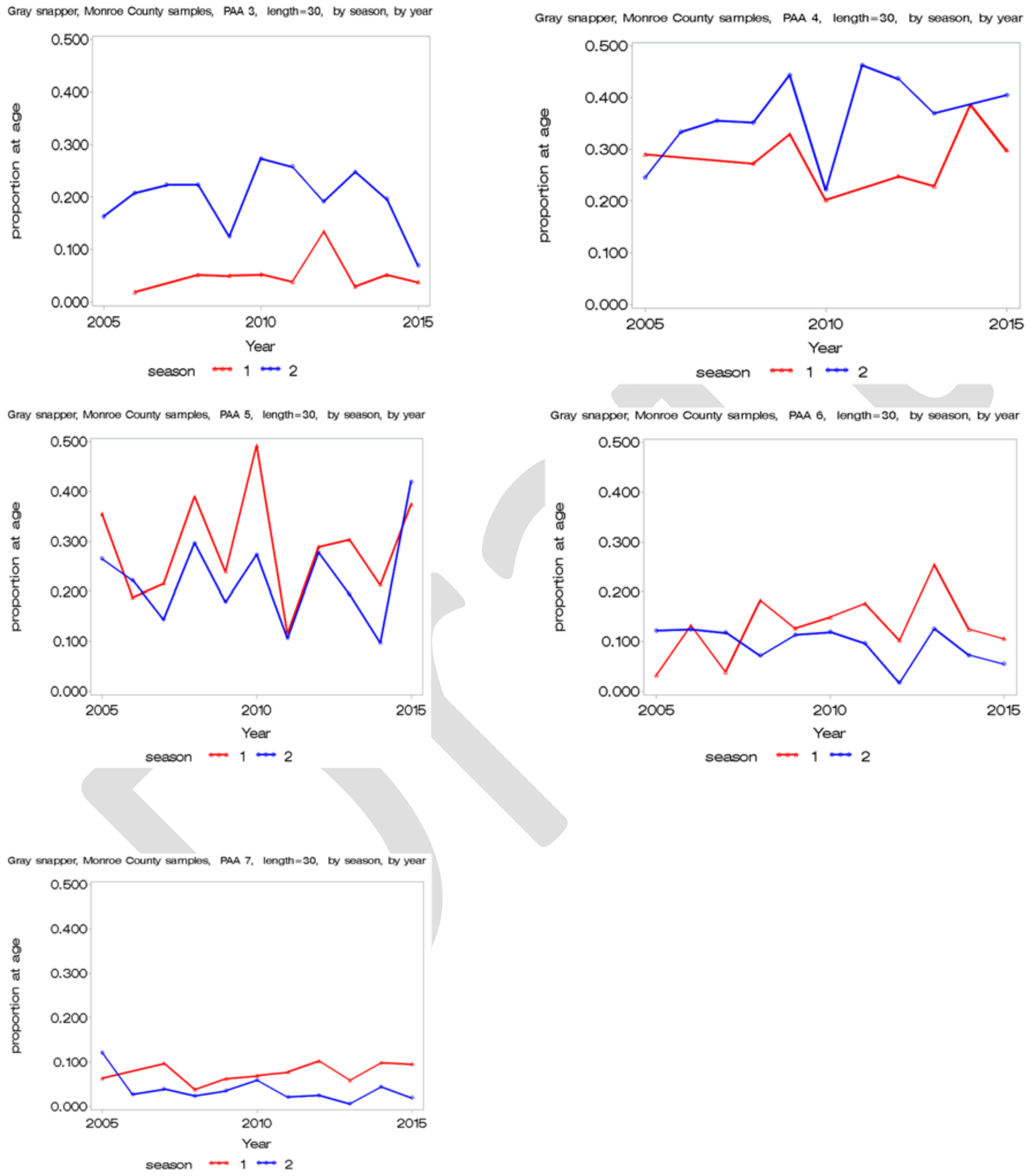


Fig 3 (c). Seasonal differences in age at lengths (ALKs) for gray snapper samples collected from Monroe County.


Gray snapper, Monroe County samples, PAA 3, length=30, by season, by year

Gray snapper, Monroe County samples, PAA 4, length=30, by season, by year

Gray snapper, Monroe County samples, PAA 5, length=30, by season, by year

Gray snapper, Monroe County samples, PAA 6, length=30, by season, by year

Gray snapper, Monroe County samples, PAA 7, length=30, by season, by year