ACCURACY AND PRECISION OF FIN-RAY AGING FOR GAG
(*MYCTEROPERCA MICROLEPIS*)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
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This thesis is dedicated to my parents, Cal and Maggie Debicella.

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The ability to age fish accurately is an essential first step for assessment of a fish population and successful resource management. The most accepted method of aging long-lived marine fish uses otoliths and is lethal. The sacrifice of large numbers of threatened fish or fish in protected areas is counter-intuitive to their conservation and may be avoidable through the use of alternative aging methods. The primary goal of this study was to assess the use of the nonlethal fin ray method for aging recreational and commercially important groupers, using gag (*Mycteroperca microlepis*) as a model species. Gag were collected from artificial reefs and live-bottom areas off the west coast of Florida, local fish markets and from fishing guides. Paired samples of otoliths and dorsal fin rays were obtained from each fish and ages were determined using cross-sections of each aging structure. Ages based on the conventional method of using otoliths ranged from 0 to 20 years old and fin ray ages ranged from 0 to18 years old.

Within-reader agreement for Readers 1 and 2 was 92% and 95% for otoliths (Average Percent Error or APE=0.6 and 1.0, respectively) and 62 and 64% for fin rays (APE=6.8 and 9.3), with agreement of ± 1 yr of 97-99% and 94-95% for otoliths and fin rays respectively. Between-reader agreement was 83% for otoliths (95% ± 1 yr) and 59% for fin rays (90% ± 1 yr). Percent agreement between otolith-based and fin ray-based ages was 56 to 62% (APE=5.9-6.2), with 87-95% agreement within ± 1 yr. Qualitative marginal-increment analysis indicated that otoliths and fin rays completed deposition of the opaque zone (relatively fast growth) at approximately the same time each year (May-August). Four main aging inaccuracies were of particular concern when aging gag using fin rays, including an occluded first annulus, identifying false annuli or checks, compaction of annuli on the edge of aging structures and placing fish into the appropriate age classes. A test of symmetry for the two methods of aging gag showed an overall systematic bias for aging gag from 0-17 years old with fin rays, which was statistically attributable to biases in aging 3 and 7 year old gag due to problematic false annuli. Age composition of gag (1 through 9, and ≥10 years of age) based on proportion-at-age in the hook-and-line landings was not significantly different between otolith-based ages versus fin-ray-based ages. A test of symmetry indicated a systematic bias in the aging that was significant only for 4 year old gag. Again, this bias was most likely due to problems with identifying checks. Further examination of both otoliths and fin rays is needed for older gag (>8 years) because otoliths from older gag were problematic and aged with less reliability than fin rays. The success in using the fin-ray aging method for gag, as a model grouper species, suggests that there is utility in further developing the method for aging other serranids, such as the threatened goliath and Nassau groupers.

CHAPTER 1
INTRODUCTION

Fisheries managers examine population parameters to determine the status of protected and exploited species. The ability to age fish accurately is an essential first step for age-based assessment of a fish population and successful resource management (Crumpton et al. 1984; Brennan and Cailliet 1989). The ability to construct an accurate age structure of a fish population is critical because many current stock assessment models in use for marine fish populations are age-structured and depend on a catch-at-age matrix (e.g., Turner et al. 2001). These models allow for mortality estimates, age-specific growth rates, and age-specific fecundity and maturity estimates. Reliability of these estimates ultimately influences the outcome of stock assessments and thereafter management approaches for a fishery. If fish are not aged accurately, the stock assessment and resultant management decisions may be biased.

Fish are generally aged by examining annual variations in the growth of hard parts, such as scales, spines, fin rays, otoliths and/or bones, including opercles, clavicles, and cleithra (Chilton and Beamish 1982; Campana 2001). The most accepted method of aging long-lived marine fish involves the use of otoliths. Otoliths are composed of calcium carbonate (calcite and aragonite) embedded in an organic matrix and assist bony fishes in balance and sound perception (Chilton and Beamish 1982; Victor and Brothers 1982). Otoliths generally contain the best permanent pattern of fish growth because calcium resorption is not known to occur from the otoliths and some deposition occurs each year (Chilton and Beamish 1982). However, the fish must be sacrificed to obtain the otoliths from the pars inferior located in the skull. The sacrifice of large numbers of threatened fish species or fishes in protected areas is counter-intuitive to their conservation and may possibly be avoided through the use of alternative aging methods.

There are several nonlethal alternative methods for aging fish, including the removal of external hard parts from fish such as scales, soft fin rays, and fin spines. Traditionally, scales have been used to age freshwater fish, as well as relatively short-lived (<5-6 years) marine fish (Beamish 1981). Although scales are an effective, nonlethal way to age rapidly growing fish with few year classes (Chilton and Beamish 1982), they often underestimate the age of fish with greater life spans (>10 years). This is a result of the annuli becoming crowded near the edge of the scale, which makes it difficult to enumerate the number of annuli in older fish (Beamish and Harvey 1969; Chilton and Beamish 1982; Dutka-Gianelli 1999). Another nonlethal method for aging fish is to use their soft fin rays or stiff spines, which can be removed and the fish released unharmed (Beamish and Harvey 1969; Mills and Beamish 1980). Fin ray sections have been used to age fishes for more than 60 years (Cass and Beamish 1983), including a variety of freshwater and saltwater species, such as white sucker (*Catostomus commersoni)* (Beamish and Harvey 1969), white sturgeon (*Acipenser transmontanus)* (Brennan and Cailliet 1989), lake whitefish (*Coregonus clupeaformis)* (Mills and Beamish 1980), lingcod *(Ophiodon elongatus)* (Beamish and Chilton 1977; Cass and Beamish 1983), walleye pollock (*Theragra chalcogrammma),* Pacific cod (*Gadus macrocephalus)* and albacore tuna (*Thunnus alalunga)* (Beamish 1981), sockeye salmon (*Oncorhynchus nerka)* and chum salmon *(O. keta)* (Bilton and Jenkinson 1969), and brown trout (*Salmo trutta)* (Shirvell 1981). These fish are primarily from cold, temperate regions where annual ring depositions are relatively wide and distinct.

The use of fin rays or spines, in particular, as nonlethal aging methods has not been thoroughly assessed using warm-temperate fish species, and is especially appealing for species that are threatened or in marine protected areas, such as goliath grouper (*Epinephelus itajara*) and Nassau grouper (*Epinephelus striatus*). White grunt (*Haemulon plumieri*), a warm-temperate species in the eastern Gulf of Mexico, has been successfully aged up to 10 years old using fin rays (Murie and Parkyn 1999). For fish less than 10 years old there was 90% agreement between ages from sectioned otoliths and ages from fin rays, while fish 10 years old or greater had their age underestimated using the fin-ray method due to accumulation of annuli on the edge of the fin rays, without periods of growth between consecutive annuli (Murie and Parkyn 1999). Gray triggerfish (*Balistes capriscus)*, another warm-temperate species from the northeastern Gulf of Mexico, have been aged up to 13 years using their single dorsal spine. The spine sections showed distinct, dark bands with agreement between readers of 98% (Johnson and Saloman 1984). These two examples suggest that other warm-temperate fish species could be aged using fin rays or spines. The general applicability of the method therefore warrants further examination for warm water species, especially for species in which there is a conflict between researchers using lethal methods to age fish and user groups concerned with conservation of these same fish populations.

Chilton and Beamish (1982) have found that the advantage of using fin rays for age determination is that it is often possible to age older fish more accurately than if scales were used, and the fish still does not have to be sacrificed. The problem of the annuli becoming crowded and indiscernible in older, slow-growing fish when aging using scales (Beamish and Harvey 1969) can also be a problem when aging fish using fin rays. However, the age at which annuli begin to accumulate on the edge of the fin ray is species-specific and tends to occur at an older age than with scales (Chilton and Beamish 1982). Annuli accumulation on the edge of fin rays may therefore occur at an age close to or beyond that necessary for determining age-specific population parameters or a stock assessment for a particular species. In addition, the interpretation of the first annulus and identifying false annuli ("checks") can be difficult in fin-ray aging. Fins can also be more difficult and time consuming to handle, store, and prepare than scales or otoliths (Beamish 1981; Chilton and Beamish 1982).

It is also important to examine different fins when aging a species for the first time to determine which fin provides the clearest pattern of growth, as this can differ from species to species. For example, walleye pollock are aged using their pectoral fin rays, whereas dorsal fin rays are used for lingcod (Beamish and Chilton 1977; Beamish 1981). In addition, it is practical to also determine at what location and level on the body of the fish to remove the fin ray so that the first annulus is retained and minimal harm is imposed on the fish. This is important because Mills and Beamish (1980) found that small whitefish regenerated a deformed fin ray after being clipped at the base of the pelvic fin, while fish larger than 22 cm did not regenerate rays at all. It may not be necessary to excise fin rays at their extreme base, which is located within the epiaxial musculature, as long as the first annulus is retained and remains recognizable at the level that the fin ray is cut. Beamish (1981) found that the best sections of fin rays for walleye pollock, Pacific cod, and albacore tuna came from 1.0-1.5 cm above the point where the ray base starts to flare. However, Bilton and Jenkinson (1969) noted when aging sockeye salmon with fin rays that the innermost ring on the section of the fin ray nearest the base was not present on sections from further out on the ray.

Whenever a fish is aged, it is vital that the method used be validated across all age classes for a particular species. Validation provides evidence that a technique is accurate and that the estimated ages are therefore as close to the true value (age) as possible. Beamish (1981) and Campana (2001) recognize several techniques commonly used to validate an aging method. Several of these techniques have been used to validate annual deposition in otoliths, including: marginal-increment analysis, where changes in the width of the growth increment at the edge of the aging structure is measured over the course of a year; chemical marking of fish that are later recaptured; and by comparing ages determined from different structures, such as otoliths, fin rays, and scales, to an aging structure that has been previously validated (Beamish 1981).

Besides validating or determining the accuracy of using fin rays to age warm-temperate fishes, it is also essential to evaluate the precision or reliability of the method. There are two potential aging inaccuracies that are of particular concern when using fin rays. First, identifying the first annulus can be problematic and can result in systematic under-aging by one year (MacFarlane and King 2001). This may not be critical for long-lived species, such as many rockfishes (*Sebastes* spp.) that live beyond 50 yrs (Leaman and Beamish 1984), but can have serious impacts on population parameters and stock assessments for short- or moderately-lived species where there might be only 4 or 5 year classes that dominate the fishery (MacFarlane and King 2001). Second, and most importantly, ages based on fin rays may underestimate the true age of older fish. The threshold age at which compaction of annuli and therefore under-aging occurs is species-specific and must therefore be estimated for each species of interest. Under-aging the older component of a population can result in a skewed age distribution, which could lead to overestimation of mortality (i.e., using a catch curve), as well as biased estimates of age-specific population parameters, such as growth, fecundity, and maturity.

There are many warm-temperate fish groups in which a nonlethal aging method would be an asset, including the Serranidae or grouper family. Various groupers comprise important recreational and commercial fisheries in Florida. Red grouper (*Epinephelus morio)* make up approximately two thirds of the overall commercial and recreational grouper landings (Goodyear and Schirripa 1993), while the remainder of grouper landings is equally divided between gag (*Mycteroperca microlepis*) and all other grouper species (Shirripa and Goodyear 1994). Total landings of gag in Florida were 3.5 metric tons (7 million pounds) in 2001. Of the gag harvested in 2001, 89% were landed on the Gulf coast and 56% of those landings were made by recreational anglers (www.floridamarine.org). Due to previous overexploitation (Turner et al. 2001), gag are regulated by annual quotas, minimum size limits, and daily bag limits (Gulf of Mexico Fishery Management Council 2001).

Members of the genera *Epinephelus* and *Mycteroperca* are particularly vulnerable to the threat of overfishing (Morris et al. 2000). The impacts of overfishing are amplified in these genera due to the tendency of many species to form predictable spawning aggregations. In particular, spawning aggregations of goliath grouper, Nassau grouper and gag have been heavily exploited. Due to low abundance, goliath grouper and Nassau grouper have been prohibited to harvest in Florida waters since 1985 and 1990, respectively (NMFS 1990a,b; Gulf of Mexico Fishery Management Council 2001). Although gag remain open to recreational and commercial fisheries in Florida, their harvest is heavily regulated as part of an annual quota, as well as by size and bag limits (Gulf of Mexico Fishery Management Council 2001). Beginning in the 1980's, the average size of gag in the Gulf of Mexico shifted toward smaller fish and the proportion of males decreased (from 17% to 1% in the last 20 years) in the population (Coleman et al. 1996). Since gag are protogynous, a severe reduction in large males in the population has the potential to limit reproduction. In addition, as females age they spawn more frequently and their fecundity increases with weight and length (Collins et al. 1996). Because of concerns that the removal of larger, older females and males has limited reproduction and subsequent juvenile recruitment, several regulations have been recently implemented for gag including harvest prohibition during the main months of their spawning season (March-April). In addition, marine protected areas have been set aside in the main gag spawning area to both protect spawning aggregations of gag as well as other reef fishes (Gulf of Mexico Fishery Management Council 2001).

Thus, gag are a suitable grouper species in which to attempt to apply a nonlethal aging method not only from a management perspective, but they are also suitable as a model species for other protected, moderately-lived, warm-temperate reef species, such as Nassau and goliath grouper, that occur in far lower abundance. In particular, gag are a moderately-aged grouper species, with a maximum age variously estimated as 22 years by Collins et al. (1987) and Harris and Collins (2000) and 28 years by Fitzhugh et al. (2003). Maximum ages of Nassau grouper and goliath grouper are either younger (16 years for Nassau grouper, Olsen and La Place 1979) or in the same approximate age range (37 years for goliath grouper, although 90% of goliath grouper were <24 years old, Bullock et al. 1992). Gag are also advantageous as a model species because ages estimated using otoliths have been validated using a variety of methods, including marginal-increment analysis (Hood and Shlieder 1992), chemical marking with oxytetracycline (Collins et al. 1996), and mark-recapture growth analysis (Schirripa and Burns 1997). This makes it possible to use otolith ages as an indirect validation of fin-ray ages if necessary. In addition, to assess the potential under-aging bias of fin-ray aging, it is necessary to sample relatively older groupers. Whereas this may still be problematic for gag, it is at least potentially feasible given that fisheries are ongoing for the species, in contrast to Nassau and goliath groupers where the fisheries are closed. Developing nonlethal methods of fin-ray aging using gag as a model grouper species may therefore be more efficient and, ultimately, of direct benefit to ongoing recovery efforts of Nassau and goliath groupers if the methods and interpretation are transferable to these protected species.

Therefore, the primary goal of the present study was to assess the use of the fin-ray method for aging a warm-temperate moderately-lived grouper, using gag as a model species. The specific objectives were to: 1) examine different fins to determine which has the clearest pattern of alternating opaque and translucent zones necessary for aging gag and estimate precision for both otoliths and fin rays; 2) test the accuracy of the fin-ray method for gag by comparing the pattern and timing of the annual deposition in fin rays and otoliths, of which the latter has been previously validated; 3) compare fin-ray ages to ages obtained using otoliths, of which the latter is considered to be the best or least biased structure in aging fishes; and 4) determine if any resulting biases or error associated with the fin-ray method of aging would result in a significantly different age composition of gag compared to an otolith-based age composition. This final objective was necessary to determine if fin-ray aging is an appropriate method that could potentially be used to age threatened groupers or groupers residing in marine protected areas, either for direct stock assessment purposes or to assess the recovery in a truncated age structure that is typical of heavily or overexploited fish stocks.

CHAPTER 2
METHODS

Fish Collection

Gag samples were collected from a variety of sources, including: 1) fish traps, spear fishing, and hook and line from artificial reefs and live bottom areas at ~15 m depth off the west coast of Florida; 2) traps and hook and line from other, unknown offshore areas of the west coast of Florida (pers. comm.; Mr. Lew Bullock, Florida Marine Research Institute; Dr. Gary Fitzhugh, National Marine Fisheries Service, Panama City, FL; and Dr. Chris Koenig, Florida State University); 3) local fish markets, which land fish from various depths and areas in the northeastern Gulf of Mexico (e.g., Northwest Seafood, Gainesville, FL); and 4) fishing guide boats landing catch in Steinhatchee, FL. All whole fish were measured for maximum total length (TL) and weighed, with filleted gag measured only for TL. Sagittal otoliths were removed from each fish, rinsed in distilled water, and stored dry until processed. Dorsal fin rays were collected from all fish, while dorsal fin spines and anal and pelvic fin rays collected from a subsample of fish. By sampling only the mid-central rays of the fin it was easier to avoid frayed or damaged rays or rays that had grown and fused together (Chilton and Beamish 1982). Dorsal fin rays 3-7 and dorsal spines II-V were clipped at the base of the pterygiophores. Excess tissue was trimmed away with scissors and then fins were stored in a coin envelope with fin rays lying flat and parallel to one another with the cut surface exposed. Fins were air dried for approximately 2-5 days or stored frozen.

Aging Structures

Sagittal Otoliths

In preparation for sectioning, the left otolith (the right otolith was used if the left was unavailable) was heat annealed to a frosted slide with hot glue. The mounted otolith was then thin-sectioned using a Buehler® Isomet 1000 digital sectioning saw (Buehler®, IL) with a diamond wafering blade (7.6 cm diameter X 0.15 mm blade width). Sections 0.5 mm thick were taken through the nucleus of the otolith along a transverse plane. Otolith sections were rinsed in ethyl alcohol and then permanently mounted on glass slides with Histomount® (National Diagnostics, GA). Sections were viewed under transmitted light (25-45x power) using a stereomicroscope. Under transmitted light, a combination of one dark opaque zone (or optically more dense band) with one translucent (clear light transmitting) zone was interpreted as a complete annulus, with the narrow opaque zone counted to determine the age (Collins et al. 1987; Hood and Schlieder 1992; Harris and Collins 2000).

Fin Rays

For final processing, dried fins were boiled in distilled water for approximately 10 minutes and the skin removed from the base of the fin rays using a soft-bristled brush. Fins that were stored frozen were thawed and then submerged in boiling water for approximately 1 minute. Skin was then peeled away from the base of the fin rays using forceps. Cleaned fin rays were then air-dried after which they were coated with Hysol® epoxy adhesive (Loctite Corporation, Rocky Hill, CT) and allowed to harden for at least 48 hours. Epoxied fin rays were cross-sectioned using a Buehler® Isomet 1000 saw with a diamond wafering blade (15.2 cm diameter X 0.5 mm blade width). Sections were cut to ~ 0.8 mm thick and were permanently mounted on glass slides using Flotexx®. Fin-ray sections were viewed with transmitted light (4-10x power) using a compound microscope (Chilton and Beamish 1982). A narrowband green filter (wavelength 550 nm) was used to enhance contrast. In fin-ray sections, each annulus also appears as a pair of alternating translucent and opaque zones. However, in fin rays it was the narrow translucent zone that was enumerated as per Chilton and Beamish (1982).

A subsample of dorsal fin rays from 9 gag ranging from age 0 to 5 was used to assess changes in the appearance and presence of annuli from proximal to distal sections. One of the 4-5 rays in each of these samples was cut where the fin ray stands free of the back of the fish to serve as a marker for the external portion of the rest of the fin rays. When the sample was aged it was possible to compare the ages of the rays below and at the level of the cut ray to examine whether any annuli (especially the first, which was laid down when the fish was smallest) were missing in the sections taken at the level where the fin ray stands free of the back of the fish.

Rays from different fins (dorsal, anal, and pelvic) and dorsal fin spines were also assessed for clarity of annuli. Sections from the various structures were taken at variable widths (0.6-1.1 mm) to determine the best section width for each structure. The structures were comparatively aged to determine which provided the most reliable, clear pattern of annuli for aging.

In addition, a subsample of 138 fin rays from age classes 1-7 were examined for the mean diameter of the first and second annulus. Diameters of the first two annuli were measured perpendicular to the axis of the inner groove of the fin ray according to the method of Chilton and Beamish (1982) (Figure 1) using a Motic® M62 capture board and Motic® image processing system (Version 1.3; Motic, Inc., Richmond B.C.).

Measurements were always taken from the section closest to the base of the fin ray for consistency. These measurements were taken to assess the possibility of a missed first annulus in older age classes. A single factor analysis of variance (ANOVA) was used to test for a significant difference between the first and second annulus among year classes by using the difference between the two annular measurements (Penha et al. 2004).

Aging Criteria

Otoliths were aged according to criteria set forth by Collins et al. (1987) and Hood and Schlieder (1992). Aging criteria for gag fin rays was developed from general fin-ray aging criteria outlined in Chilton and Beamish (1982), with modifications to distinguish what an annulus looks like in a fin ray compared to an otolith. Aging criteria were initially developed based on an examination of a subsample of 50 fish previously aged by otoliths; these fish represented the complete size range of fish collected. A second attempt to age fin rays blind (without a priori knowledge of the otolith-based age) was then made. Aging criteria were subsequently adjusted to consistently distinguish where the first annulus was located in fin rays, as well as which marks were true annuli versus checks or false annuli. A photographic reference was made for paired otolith and fin-ray sections. This reference guide included photographs of some common difficulties in aging fin rays. The reference guide and a practice set of otoliths and fin rays were reviewed by both readers prior to aging. Fin rays were always aged before otoliths, since the otolith age was considered the best age estimate based on validation of annual ring deposition by marginal-increment analysis and mark-recapture studies (Collins et al. 1987; Collins et al. 1996; Hood and Shlieder 1992). Precision and accuracy were determined for a primary reader (JMD), as well as a secondary reader (DJM).

Accuracy and Age-Class Assignment

Quantitative marginal increment analysis was performed on otoliths to validate annual deposition for the "best" aging structure. The 4 year old age class was used for this analysis because it was the only age class with an adequate sample size for most months. The marginal increment in each otolith was measured on the proximal medial surface on an axis dorsal to the sulcus, since this was the clearest readable area of the sectioned otolith for gag. These measurements were made using the image capture system (Figure 2A). In particularly older gag, annuli on the dorsal area of the otolith were more easily distinguishable than on the ventral area (Figure 2B). In this study, otoliths were considered by convention to be the "best" aging structure and were assumed to be 100% correct in estimating the age of gag in comparative analyses with fin-ray ages.

To validate the accuracy of the fin ray and otolith aging methods, qualitative marginal-increment analysis was performed on both otoliths and fin rays independent of one another. Qualitative marginal-increment analysis on otoliths and fin rays was conducted by assessing if a sample had an opaque or translucent zone on the edge. An opaque zone in otoliths was considered "on the edge" if it appeared near the sulcus (Figure 3A). A translucent zone in fin rays was considered to be "on the edge" if it appeared in both the faster growing, oblique area of the fin ray, as well as some part of the slower growing, outer portion of the fin ray (Figure 3B). Both the timing and number of minima present in the qualitative marginal increment for otoliths were graphically compared to the qualitative marginal increment for fin rays to determine whether the deposition of material on the edge of the aging structures occurred in a synchronous or differential manner.

Age classes were assigned for each aging structure using the conventional birthdate of January 1 (Chilton and Beamish 1982). With establishment of the aging criteria, all fin rays and otoliths were read at least twice by the primary reader (JMD). As the alternative aging method, fin rays were always read first and independently of the paired otolith. All readings were independent of the age assigned in previous readings, and there was a minimum of 2 weeks between readings. In addition, no knowledge of the size or collection date of the fish was used when reading the aging structures. When age estimates for the primary reader were in agreement, then that age was considered to be the resolved age. If the two age estimates disagreed, then the structure was aged again independently of the first two readings. A resolved age was then accepted if two out of three independent readings agreed. If two out of three ages did not agree, a fourth read was conducted and either the median age or an age that was agreed upon in two out of four readings was used as the resolved age for the structure. If a resolved age could not be determined for a structure using these criteria then the structure was deemed unreadable.

Precision of Age Estimates

To determine between-reader precision, a secondary reader (DJM) estimated ages for a randomized age-stratified subset of 111 fin rays and otoliths. Resolved ages were obtained in the same manner as for the primary reader and all age estimates were based on the same aging criteria for defining an annulus as provided to the primary reader.

Precision (reproducibility) was estimated using the first two readings by determining percent agreement (Sikstrom 1983), coefficient of variation (CV) (Kimura and Lyons 1991), and average percent error (APE) (Beamish and Fournier 1981). All measures of precision were calculated within reader, between readers and between aging structures. Measures of percent agreement, coefficient of variation, and average percent error are commonly used to assess precision in aging methods and therefore facilitated comparison with other studies. Lower values for CV and APE represent greater precision. Between-structure precision was also determined by calculating the concordance correlation coefficient (Lin 1989, 2000; Murie and Parkyn 2002). The concordance correlation ranges from –1.0 to 1.0, with 0 representing no reproducibility. The concordance correlation has been determined to be more robust than the coefficient of variation, paired t-tests, Pearson's correlation coefficient and the least squares approach (Lin 1989; 2000).

Comparative Age Estimates Using Fin Rays versus Otoliths

The comparison between resolved otolith and fin ray ages was visualized by plotting the fin-ray age as a function of the sectioned otolith age for each fish. A line denoting the ideal 1:1 relationship was used for reference (Beamish and Harvey 1969). Age estimates from otoliths and fin rays were also compared quantitatively using a test of symmetry to determine systematic aging differences between structures (Bowker 1948; Hoenig et al. 1995). If a significant difference was detected, then the test of symmetry was repeated assuming symmetry for the most extreme cases until no bias could be detected (Hoenig et al. 1995).

In addition to direct tests of any difference between the two aging methods, an applied test of the fin-ray method was conducted by comparing the proportions at age of a gag population using otoliths versus fin rays. Expected proportions were based on the Fitzhugh et al. (2003) hook-and-line data from 1997-2000, as this most closely resembled the time period and gear from which the current studies' samples were collected (original data obtained from Dr. Gary Fitzhugh, NMFS, Panama City, FL). Gag older than 10 years were pooled together into a 10+ age group, based on the catch-at-age matrix used in current gag stock assessment (Turner et al. 2001). A random age-stratified subsample from all available otoliths was then drawn to match the expected proportions. Fin rays that were paired with the drawn otolith subsample then formed the basis of the proportion at age based on the fin-ray method of aging. The age compositions (number in each age class) for each aging method were compared using a Wilcoxon Signed Rank test to determine if there was any significant difference in the estimation of gag age structure between fin rays and otoliths. To detect any systematic bias in fin-ray aging within the estimated age structure, a test of symmetry was also performed on the subsample of fin rays and otoliths. If a significant difference was detected, then the test of symmetry was repeated assuming symmetry for extreme cases until no bias could be detected (Hoenig et al. 1995).

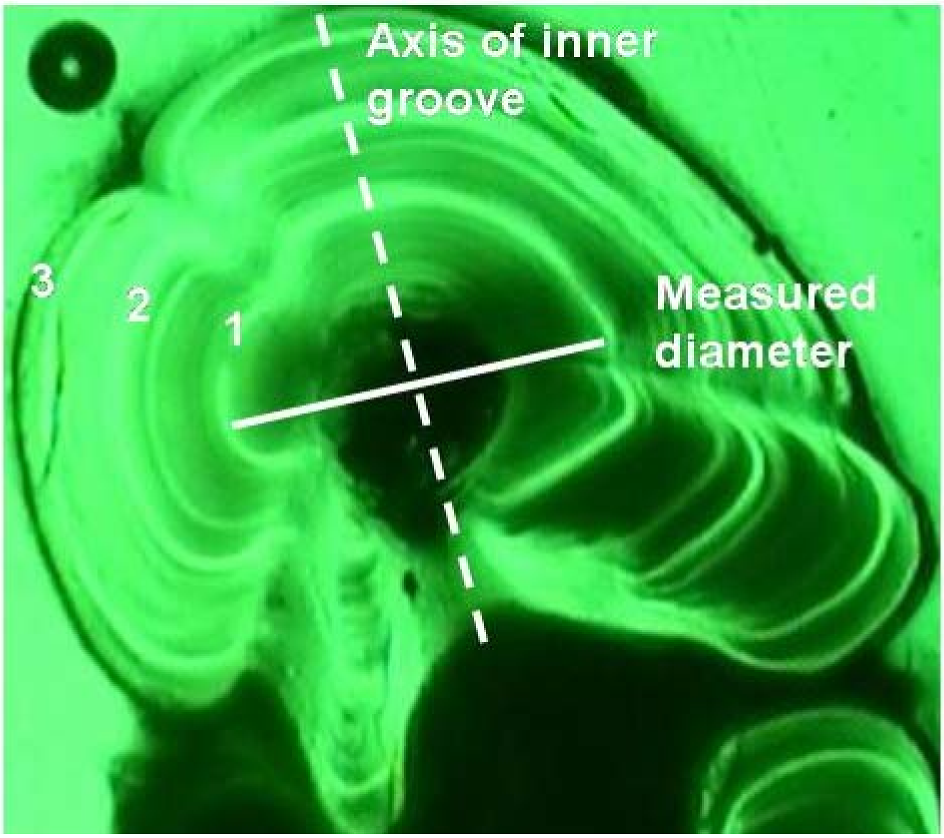


Figure 1. Mean annular diameter of the first and second annulus was taken by measuring perpendicular to the axis of the inner groove of the base of a dorsal fin ray of gag. Translucent zones for fin rays are enumerated, labeled here as 1, 2, and 3.

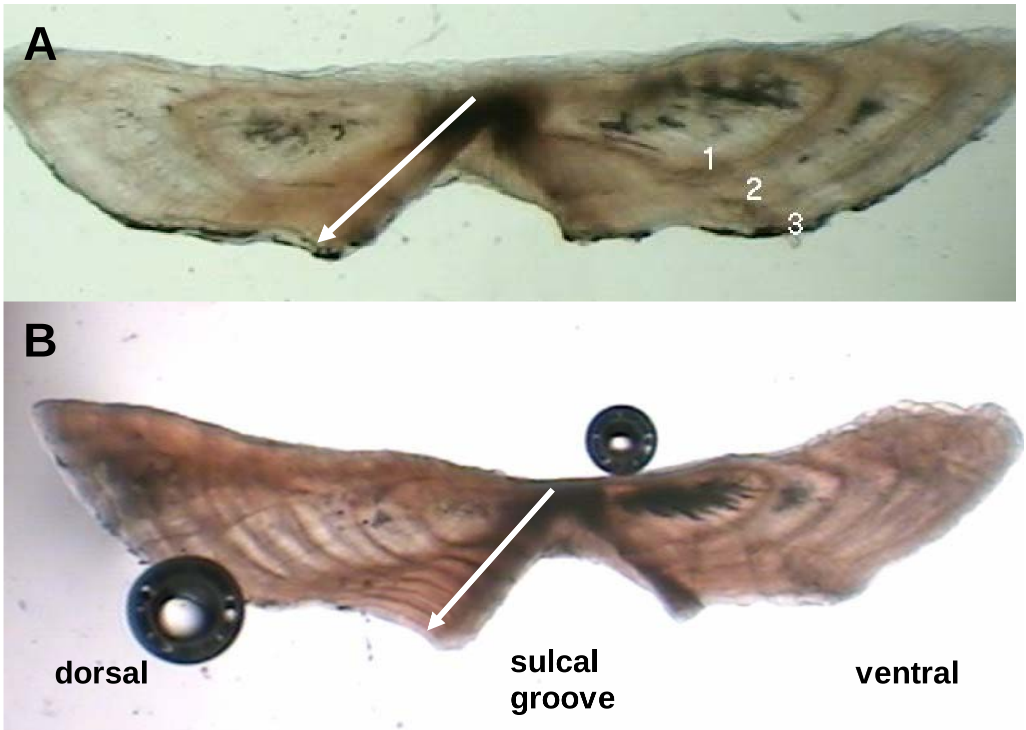


Figure 2. Cross-section of a sagittal otolith from a: A) juvenile (3-yr old) gag showing dorsal and ventral areas of the otolith relative to the sulcus; and B) older (7-yr old) gag showing annuli on the dorsal area to be clearer than on the ventral area. Marginal increment was therefore measured on the dorsal portion (adjacent to the arrow) for all otoliths.

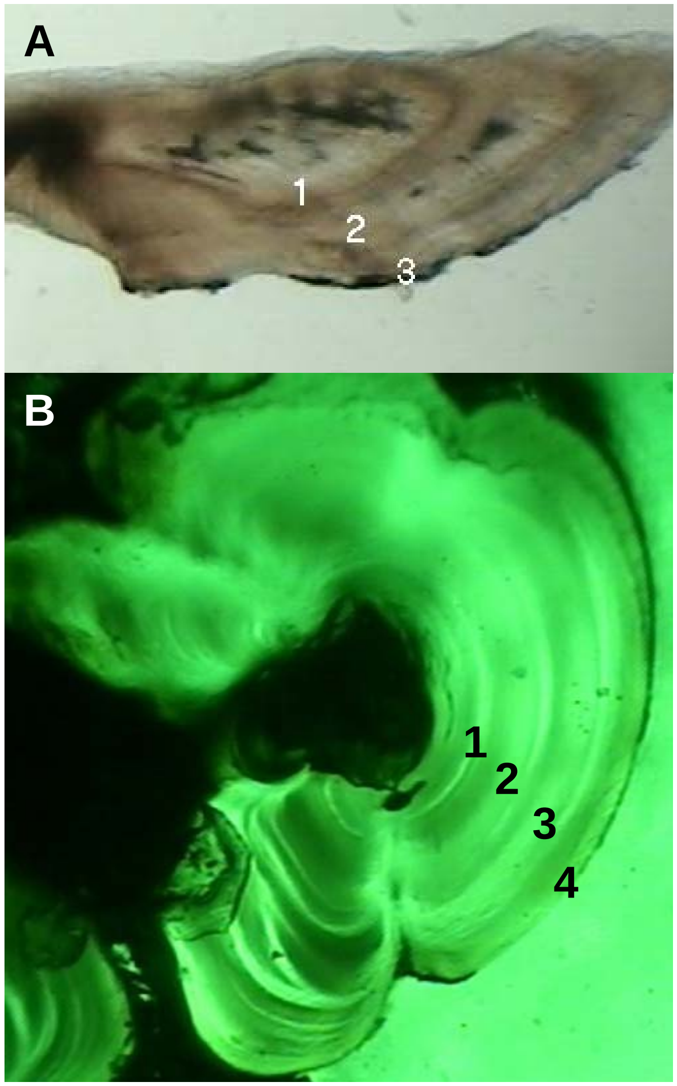


Figure 3. Position of an annulus on the edge in: A) a gag otolith where the annulus is visible along the outer edge of the otolith and near the sulcus; and B) a dorsal fin ray of gag where the annulus is visible on the fast-growing, inner area of fin ray and part of the slow-growing, outer portion of the ray. Fish in A) and B) are not the same.

CHAPTER 3
RESULTS

Fish Collection

A total of 461 paired fin ray and otolith samples were collected from gag grouper from the northeastern Gulf of Mexico (365 from fish traps, spear fishing, and hook and line; 96 from local fish markets sampling the commercial fishery). Aging structures were collected from gag ranging in size between 257 mm and 1265 mm TL (n= 437 fish) (Figure 4). Paired aging structures from 24 gag were used without any associated size data.

Aging Structures

Sagittal Otoliths

Sectioned sagittal otoliths of gag were clear to read along the medial area dorsal and ventral to the sulcus in younger fish, but only along the dorsal portion of the sulcus in relatively older fish (Figure 2A and 2B). Under transmitted light, the nucleus of the otoliths appeared opaque dark brown and optically dense. Each subsequent annulus consisted of a pair of one narrow dark opaque zone and one broad relatively translucent zone. Narrow opaque zones were enumerated to determine the number of annuli present in the otolith for consistency with other studies (Collins et al. 1987; Hood and Schlieder 1992; Harris and Collins 2000).

Fin Rays

Initial observations of the dorsal fin rays and spines, pelvic fin rays, and anal fin rays indicated that dorsal fin rays and dorsal fin spines had the clearest sections and were relatively easier to read, both due to their clarity and size. Pelvic fin rays were readable but very small in cross-section with crowded annuli, and anal fin rays were not readable beyond age 4. Preliminarily, examination of a small (n=22) subsample of otoliths, dorsal fin rays and dorsal fin spines indicated that percent agreement between dorsal fin rays and otoliths was slightly greater (71%) than agreement between dorsal fin spines and otoliths (65%) for fish ranging in age from 3-7 years. Dorsal fin rays were used for all further analysis in this study because they were easier or as easy to read as fin spines and more fin ray samples were available than fin spine samples.

Dorsal fin ray sections removed from where the fin ray stands free of the dorsum of the fish provided clear, readable sections, while still retaining all annuli (n=9, ages ranging from 0-5). No annuli were found to be missing in rays removed and sectioned at the base of the dorsal fin versus rays sectioned just above the pterygiophores (Figure 5A and B). However, as sections proceed distally up the fin ray, above the level of the dorsal surface, the first year becomes increasingly difficult to identify (Figure 5C). This is because it becomes less distinct and the sinus of the blood vessel begins to acquire an irregular shape.

For a representative subsample of gag aged 2-7 years, mean diameter of the first fin ray annulus averaged 0.56 mm (± 0.01 mm SE, range of 0.35-0.82 mm, n=118) and 0.81 mm (± 0.01 mm, range of 0.52-1.12 mm, n=118) for the second fin ray annulus. A single-factor ANOVA detected no significant difference among gag age classes in the difference between the first and second annular diameter measurements (F=1.63, n=118, p=0.157) (Figure 6). This indicated that the first and second annuli did not converge in older fish.

Aging Criteria

In general, fin rays were more difficult and time consuming to prepare and interpret than otoliths. In fin rays, annuli that were enumerated appeared as translucent bands on an opaque background (Figure 1). Viewing the edge of the fin ray was facilitated by use of phase contrast on a compound microscope, which also helped to eliminate checks (Figure 7).   Annuli were counted beginning from the central core of the fin ray, outwards toward the edge. The oblique, inner portions of the fin ray were the fastest growing areas where annuli appeared earlier than the outer edge of the fin ray, which was a relatively slower growing area.

Three impediments to aging were of particular concern: 1) an occluded first annulus; 2) identification of checks or split rings; and 3) enumeration of compacted annuli in older fish. In some dorsal fin rays, the first annulus was partially obscured by the core of the ray. If the fin ray core appeared irregular (not round), then the first annulus was usually indistinct. In these cases the annulus was visible typically at least partially outside the core and appeared as a light, translucent area (Figure 8A).

Checks, or false annuli, were present on most of the fin ray sections from gag. Often annuli were composed of more than a single ring, with all rings associated with one annulus extending around the entire ray. It was therefore important to view all fin ray sections for a fish (~9-15) to determine if certain rings always extended around the entire ray. If the rings merged together or were not complete in the majority of sections the ring was considered a check. The 2nd, 3rd, and 4th annuli in gag fin rays were often split into two very distinct rings for each annulus (Figure 8B). This was determined by comparing fin rays that had these types of checks to the otoliths from the same fish. It was also difficult to follow an annulus onto the oblique, inner portions of the ray, as the annulus often appeared to split in this area (Figure 8C).

Annuli were relatively widely spaced for the first few years and then became more closely spaced in fish aged greater than 5, and became compacted (tightly aggregated) on the outer edge of the ray in fish aged older than 6 (Figure 9A). Once annuli began compacting on the edge they appeared as thin but discrete translucent lines rather than broader bands consisting of multiple checks. Annuli appeared to compact more along the slower growing outer edge of the fin ray and they were sometimes easier to differentiate in older fish along the internal, oblique edge. Annuli on the internal edge do not appear to begin compacting before age 10 (Figure 9B).

Accuracy and Age-Class Assignment

For quantitative marginal-increment analysis of otoliths by age class, sample size throughout most months was only adequate for 4-year old gag. The observation of only one minimum in the marginal increment over a 12-month period validated the deposition of one annulus (the opaque zone) per year in this age class (Figures 10). Opaque zone completion in otoliths occurred from June-August, at which time the average marginal increment was at a minimum.

Qualitative marginal-increment analysis for gag otoliths from 2-5 year old fish showed the same trend as quantitative marginal-increment analysis from the 4 year old gag (Figure 11A). Including multiple age classes in the qualitative marginal-increment analysis was feasible because these otoliths did not need to be measured, as is the case with quantitative marginal-increment analysis, and this allowed an increase in the sample size to n=306. Translucent zones for otoliths were at a minimum for both quantitative and qualitative analyses from June through August.

Similarly, qualitative marginal-increment analysis for gag fin rays was done for 2-5 year old gag (n=247), with the difference in sample sizes between qualitative marginal-increment analysis for otoliths and fin rays due to lower numbers of fin rays having a resolved edge. As with otoliths, the translucent zone in fin rays was at a minimum from June through August, thereby indicating that the opaque zone was being deposited during this time (Figure 11B).

Therefore, based on a birthdate of approximately February/March (Hood and Schlieder 1992) and completion of the deposition of the opaque zone beginning approximately June 1st, age classes for otoliths were assigned as follows:

1. Fish collected from January through May were advanced one age class if the otolith had a significant amount of translucent zone (almost complete based on the previous interannular distance), since the fish would be one age class older within that same year. If there was an opaque annulus on the edge during this period, the age class would not be advanced because it would have to be assumed that the annulus had started to form early relative to the majority of fish.
2. Fish collected from June through December were kept in the same year class as the number of opaque annuli, unless the fish were collected between June and August and had a large amount of translucent zone growth on the edge. In this latter case, the age of the fish was advanced by one age class because it would have to be assumed that the fish was either in the process of laying down an opaque annulus or was to deposit an opaque annulus later than an average fish.

Qualitative marginal-increment analysis for fin rays revealed the translucent zone (i.e., the enumerated annulus) to be on the edge over an extended period of time (Figure 11B). Translucent zones begin to be deposited in July and were not complete until the following spring, during April and May. An opaque zone was completed primarily between June and August.

The timing of the appearance and duration of the translucent and opaque zones in fin rays indicated that they were being laid down at approximately the same time of the year as the translucent and opaque zones in otoliths. The difference in interpreting annuli between the two structures was therefore based on enumerating opaque zones for otoliths and translucent zones for fin rays. Therefore, resolved ages for fin rays were assigned as follows:

1. Fish collected from January through May with a translucent zone on the edge were kept in the same age class as the number of annuli (number of translucent zones), unless there was significant zone of opaque growth. In this latter case, the age class of the fish was advance one year because it would have to be assumed that these fish were taking longer than average to form the translucent zone.

2. Fish collected from June through December with an apparent opaque zone past the last translucent annulus were kept in the same age class as they were aged.

3. Fish collected from August through December had one year subtracted from their ages if they had a translucent annulus on the edge, since this would represent the start of the slower growth period for that year continuing into the next year (the relatively faster opaque growth period for the present year had already occurred).

Precision of Age Estimates

Overall, annuli on otoliths (opaque zones) were easier to identify and interpret than annuli on fin rays (translucent zones), except for older gag. Resolved ages of gag determined using otoliths ranged from 0 to 17 years with 0% of the otoliths illegible for Reader 1 and 1% requiring a fourth read. Reader 2 had resolved ages ranging from 0 to 20 yrs with 0% illegible and 1.8% of otoliths requiring a fourth read to obtain resolved ages. Resolved ages of gag determined using dorsal fin rays ranged from 0 to 16 years with 5% illegible by Reader 1 and 3% requiring a fourth read. Reader 2 had resolved fin ray ages ranging from 0 to 18 years with 0.9% deemed unreadable and 9.0% requiring a fourth reading for resolution.

Within-reader measures of precision for otoliths and fin rays showed that for both readers, precision was higher with otoliths than with fin rays (Table 1). For Reader 1, 92% of otoliths (APE=0.96) and 62% of fin rays (APE=4.85) were consistently read with no difference. However, 99% of otolith readings were within ±1 year and 94% of fin ray readings were within ±1 year of each other. Within-reader precision was consistent for both Reader 1 and Reader 2 for both otoliths (92% and 95%, respectively) and fin rays (62% and 64%, respectively). Scatter plots comparing the first two otolith readings indicated that within-reader precision for both the primary and secondary reader was much more variable for gag greater than 8 years of age than for gag <8 years old (Figures 12A and 12B). Although it was difficult to quantify this difference due to the small number of gag sampled that were >8 years of age, both readers found it difficult to enumerate opaque annuli along the normal reading axis on the sulcus in otoliths of older gag. Opaque annuli in these older fish were extremely thin, as if drawn by a light pencil line, and had to be counted by moving from one area to another on the sectioned otolith. Qualitatively, Reader 1 observed a large amount of translucent zone material at the edge of the otoliths in these older gag, without any apparent opaque zones, and therefore concluded that she was underaging older gag using otoliths. In contrast, scatter plots comparing the first and second fin ray readings for either reader (Figures 12C and 12D) indicated more overall variability in the precision of the readings but did not indicate that this variability increased with gag age. Both readers were more consistent in their aging of older gag using fin rays than using otoliths.

Between-reader measures of precision based on resolved ages were also higher for otoliths than for fin rays (Table 2). Scatter plots of resolved otolith ages for Reader 1 and Reader 2 indicated that Reader 1 aged gag >8 years old, in general, to be younger than the same fish aged by Reader 2 (Figure 13A). Fin ray ages differed between readers by as much as 4 years, but were within ±1 year of each other in 90% (APE=6.57) of all fin rays aged by both readers (Table 2). In general, resolved fin ray ages by Reader 1 were younger than for resolved ages for the same fish by Reader 2 (Figure 13B).

Comparative Age Estimates Using Fin Rays versus Otoliths

Fin-ray ages agreed exactly with otolith ages 62% (Reader 1) and 58% (Reader 2) of the time, with agreement ±1 year much higher (87-95%) (Table 1). Lin's concordance correlation coefficient between otoliths and fin rays indicated a near perfect concordance of age estimates from the two techniques (r_c>0.93 for both readers). Other measures of precision such as CV and APE were, in general, comparable to those calculated for within-reader agreement of fin ray ages.

The majority of fish age estimations by Reader 1 fell on the 1:1 line for fish < 4 years old (Table 2, Figure 13A). For fish between 4 and 9 years old, the fin ray method began to consistently underestimate the age of the fish when compared to the otolith method. After age 9, fin ray ages showed no systematic bias towards underaging (Figure 14A). A test of symmetry (Hoenig et al. 1995) for all age classes (ages 1-17) rejected the null hypothesis of symmetry about a main diagonal that indicates a 1:1 relationship of otolith ages to fin ray ages (χ^2 = 52.77, df = 26, p<0.001). However, inspection of the individual χ^2 components of the symmetry table revealed that the bias was attributed to 3 and 7 year old gag being underaged by 1 year (i.e., very high χ^2 components). If it is assumed that aging inaccuracies of 1 year for 3 and 7 year old gag are symmetric and the test of symmetry is rerun on this basis, then the bias associated with aging gag using fin rays becomes nonsignificant over 1-17 years (χ^2 =32.77, df=26, p>0.10).

Age composition based on otolith ages and paired fin ray ages using proportions from hook-and-line catch at age given in Fitzhugh et al. (2003) showed no significant difference in the number of fish in each age class between an otolith-estimated age composition and a fin ray-estimated age composition (Figure 15) (Wilcoxon Signed Ranks test, two-tailed, n=7, T+=17, p>0.50) (Table 3). A test of symmetry revealed bias associated with aging gag using fin rays (χ^2 = 26.3, df=12, 0.005<p<0.01) for the subsample drawn according to the Fitzhugh et al. (2003) catch at age proportions. However, closer inspection revealed that the bias was mainly attributed to overaging of fin rays by one year for 4 year old gag. If it is assumed that aging inaccuracies for 4 year old gag are symmetric and the test of symmetry is performed again, then there was no bias associated with aging gag using fin rays (χ^2= 13.3, df=11, p>0.25).

Table 1. Measures of precision within-reader, between-readers, and between aging structures for otoliths and fin rays, where CV=coefficient of variation, APE=average percent error, r_c=Lin's concordance correlation, and n=sample size.

	% Agreement (±1 yr)	CV	APE	r_c	n
Otoliths					
Within Reader					
Reader 1	92 (99)	1.36	0.96	0.97	460
Reader 2	95 (97)	0.84	0.59	0.99	110
Between Readers	83 (95)	3.31	2.34	0.96	110
Fin rays					
Within Reader					
Reader 1	62 (94)	6.86	4.85	0.93	453
Reader 2	64 (95)	9.28	6.38	0.97	110
Between Readers	59 (90)	9.30	6.57	0.96	110
Between Structures					
Reader 1 (all data)	62 (93)	8.41	5.94	0.93	453
Reader 2	58 (95)	8.92	6.12	0.98	110
Reader 1	56 (87)	8.79	6.21	0.95	110

Table 2. Percent agreement between ages estimated from otoliths and dorsal fin rays relative to the otolith-assigned age for Reader 1 (n=453).

Age Class	**Number of Fish (n)**	**% Agreement Between Structures**	**% Agreement Between Structures ± 1 year**
0	7	86	100
1	51	88	100
2	34	82	94
3	119	75	98
4	87	62	94
5	60	50	98
6	32	41	84
7	41	34	83
8	12	16	67
9	3	0	33
10	1	0	100
11	1	0	0
12	1	0	0
13	1	0	0
14	1	0	100
15	1	0	0
17	1	0	100

Table 3. Catch-at-age differences in gag aged using either otoliths or fin rays. Proportion-at-age is estimated from hook-and-line caught gag in the Gulf of Mexico based on Fitzhugh et al. (2001). Number of gag in each age class (1 through 10+) using the otolith aging method is based on a random age-stratified subsample from the present study that represents the proportions from Fitzhugh et al. (2001). Number of gag in each age class using the fin ray aging method corresponds to placement of gag into an age class based on the assumption that the otolith-based aging method provides the true age.

		Age Class									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10+**
Proportion-at-age (Fitzhugh et al. 2001)		0.01	0.04	0.15	0.36	0.18	0.12	0.06	0.02	0.01	0.04
Aging Method	**Otolith**	1	7	30	70	36	24	12	5	2	7
	Fin Ray	1	10	36	50	43	25	11	2	2	7

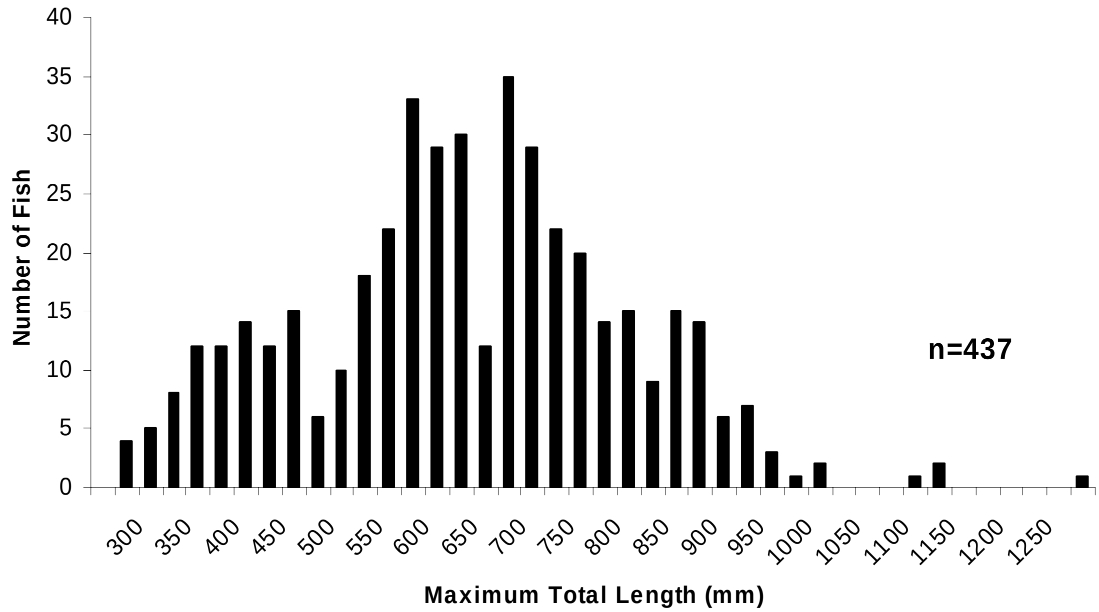


Figure 4. Length frequency for all gag used in the present study. Samples where TL was unavailable were excluded (n=24).

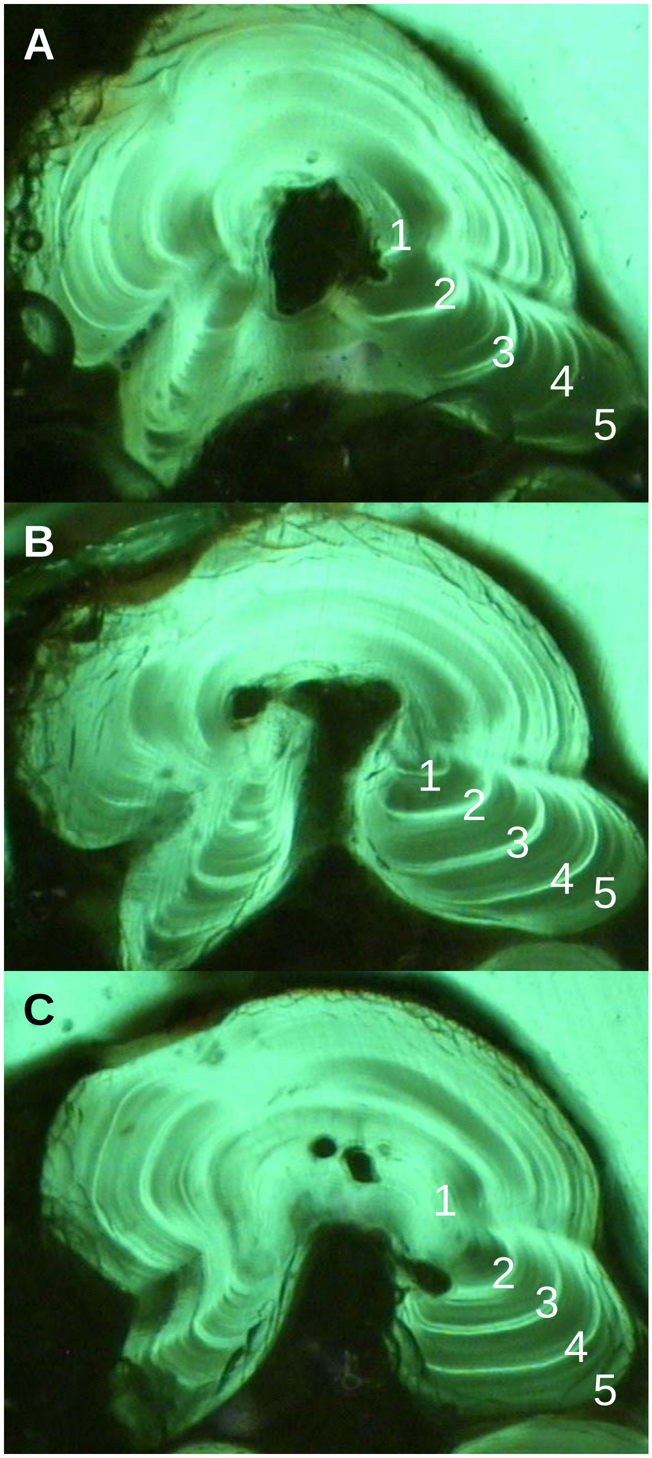


Figure 5. Annuli visible in basal versus distal sections of fin ray from an individual gag: A) basal section from just above pterygiophore on a 5-yr old fish; B) section from a ray clipped level with the back of the gag; and C) section from ~ 2.4 mm distal on fin ray of the same fish, where first annulus is present but more difficult to identify.

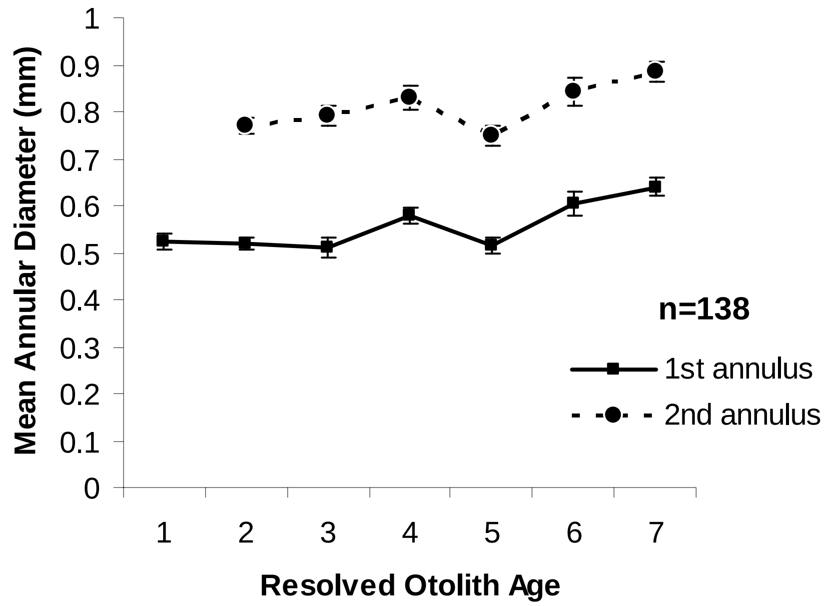


Figure 6. Mean (±SE) diameter of the first and second annuli of fin rays of gag for each age class determined by resolved otolith age.

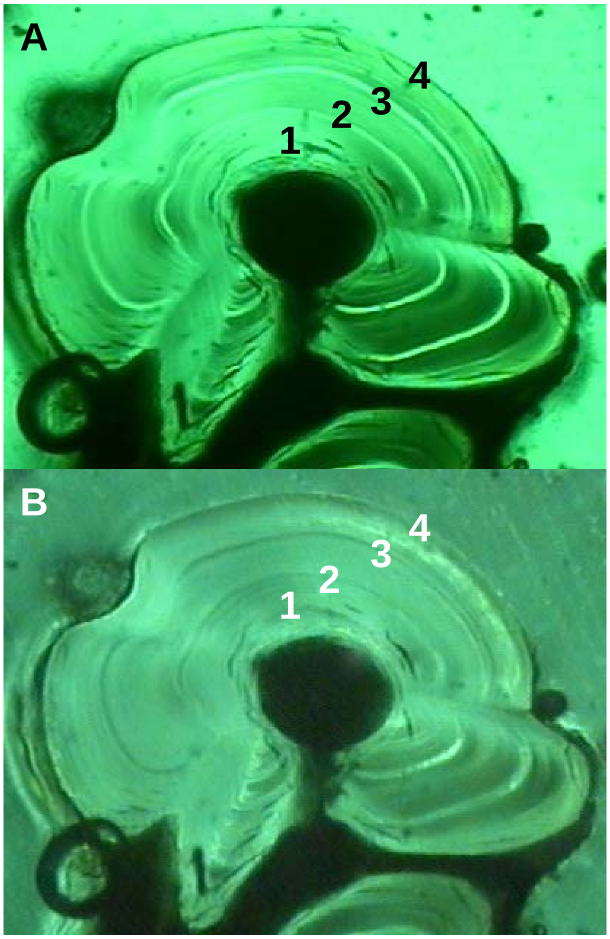


Figure 7. Picture of a gag dorsal fin ray under A) bright field (Köhler illumination) and B) the same fin ray under phase-contrast illumination. When viewed under phase contrast it was easier to determine if the annulus was on the edge or whether there was any opaque growth beyond the ultimate annulus.

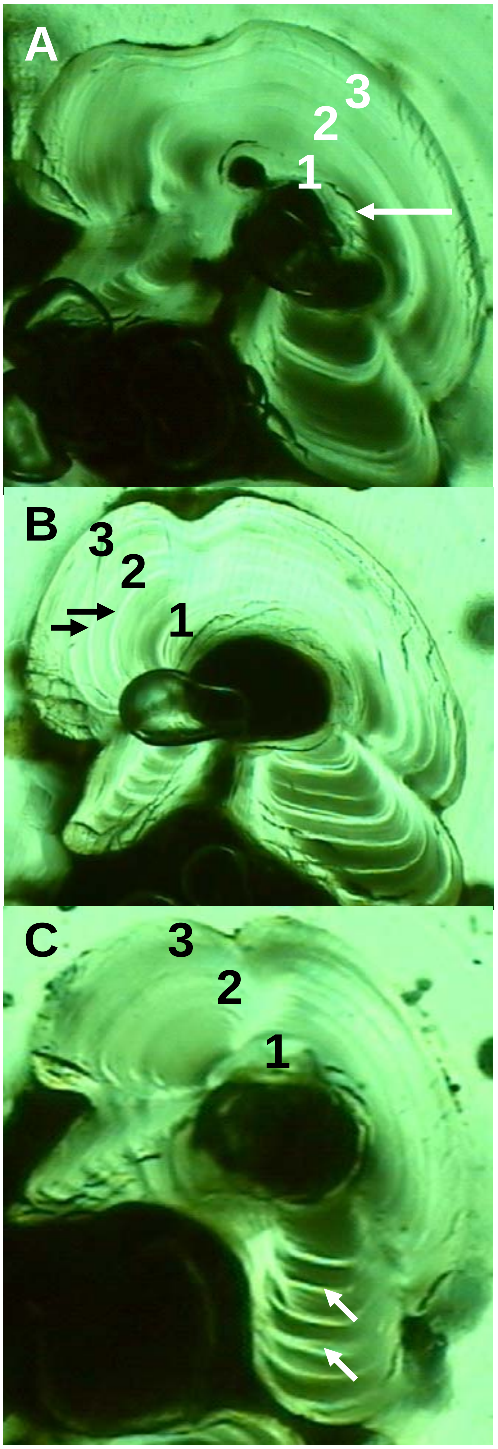


Figure 8. Cross-section of dorsal fin rays of gag: A) showing how the first annulus can be partially occluded by blood vessels that run through the center of the fin ray (3-yr old fish); B) showing distinctly split rings for 2nd annuli. This 3-yr old fish could be mistakenly aged as a 5 or 6 yr old fish; and C) showing annuli splitting into two distinct rings on oblique inner portion of fin ray.

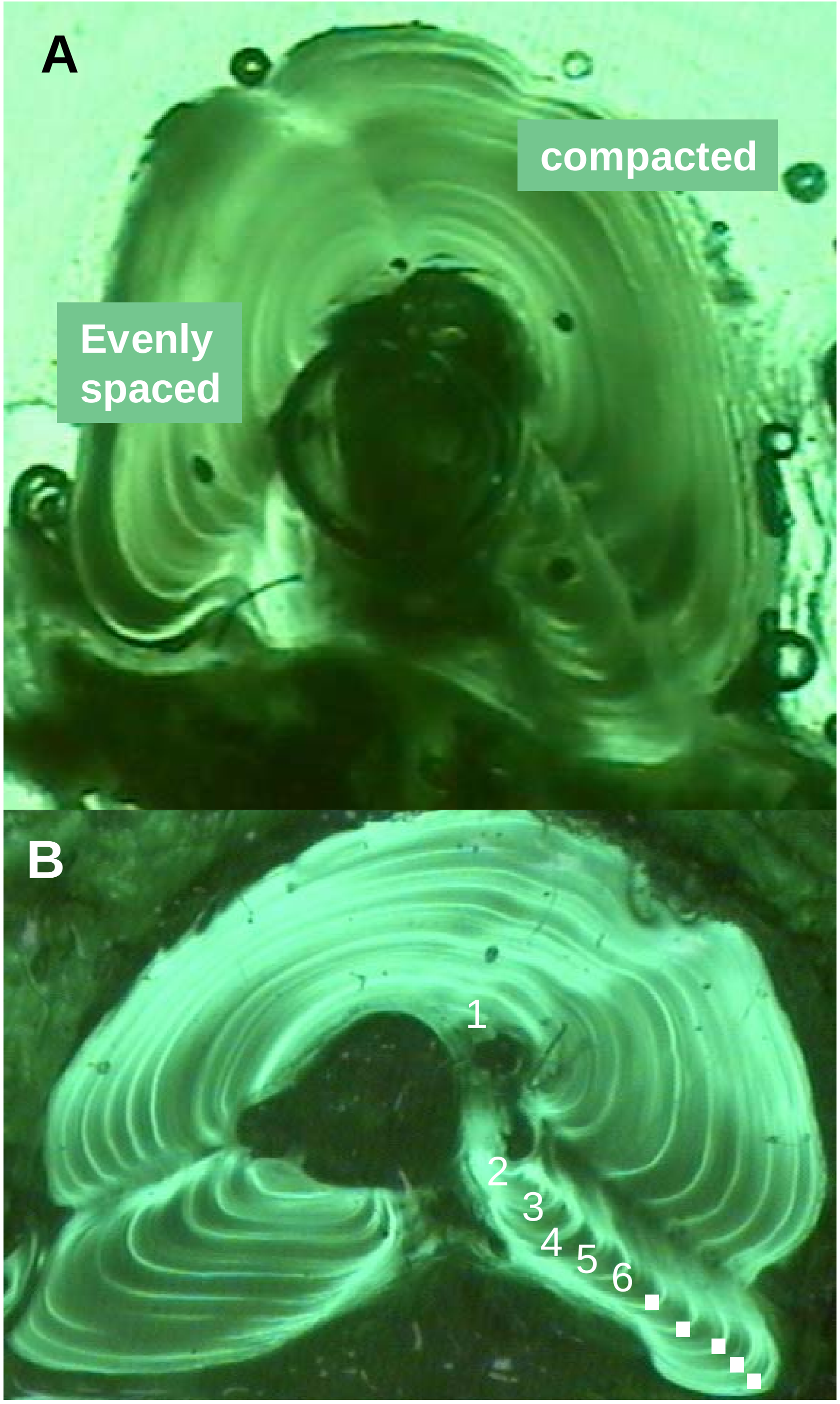


Figure 9. Cross-section of a dorsal fin ray of a gag showing: A) annuli compacting on the outer edge of the fin ray in the 5th and 6th years, whereas annuli are still evenly spaced near inner, oblique portions of the fin ray; and B) an 11-yr old gag with very clear, evenly spaced annuli on the inner, oblique edge. Annuli are slightly more difficult to distinguish on the outer portion of the ray.

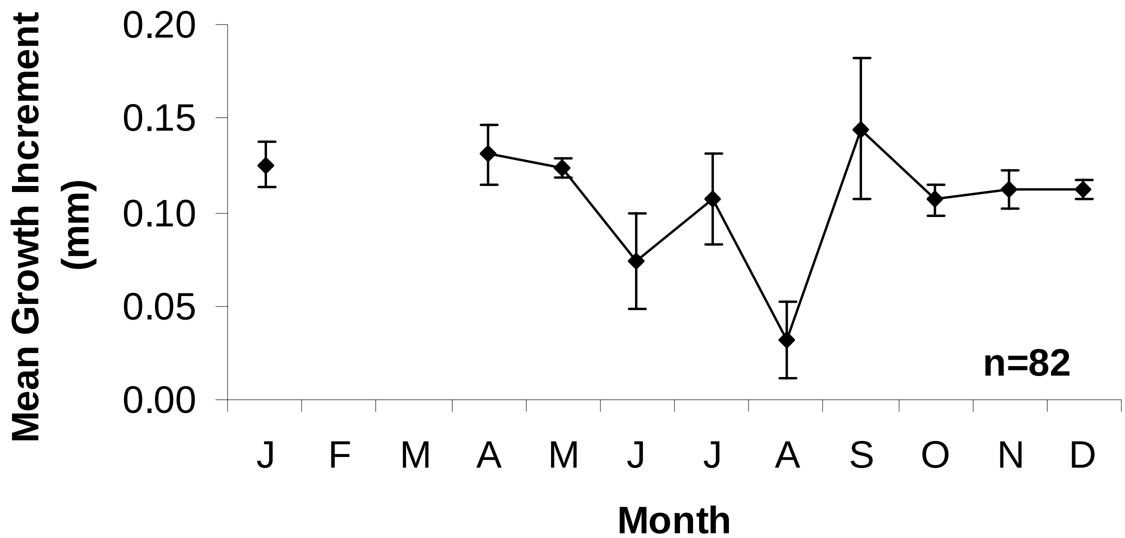
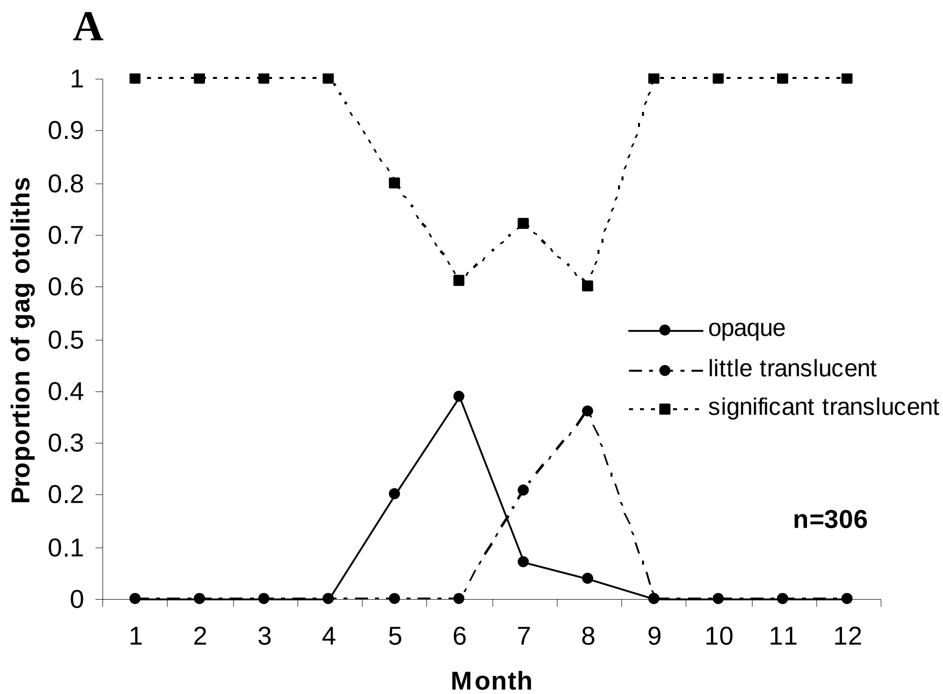


Figure 10. Mean marginal translucent growth increment (±1 SE) of age-4 gag otoliths over a 10-month period (April to January).



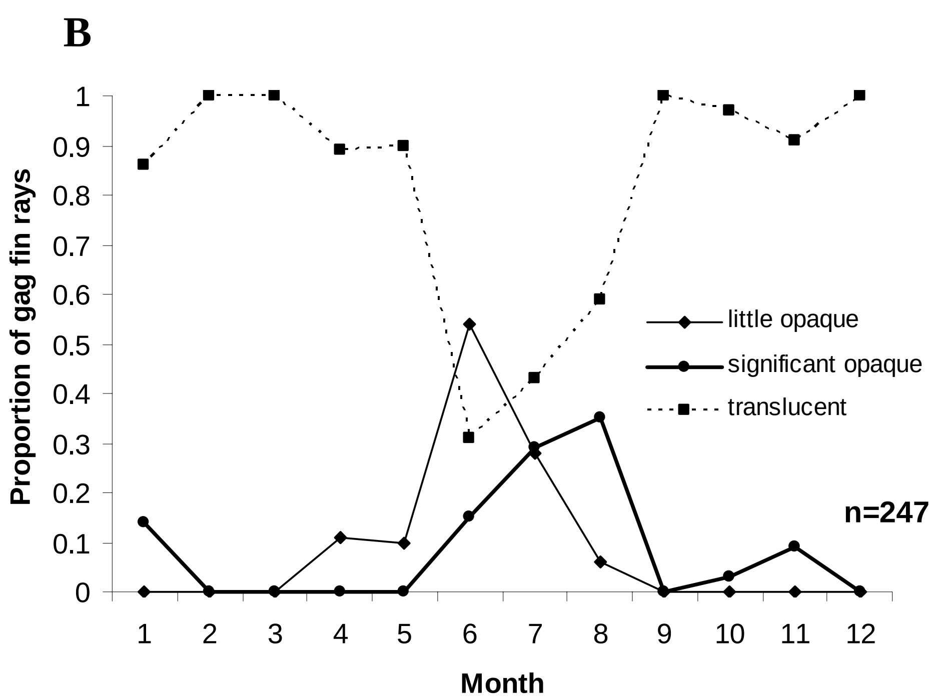


Figure 11. A) Qualitative marginal-increment analysis of the amount of translucent zone growth beyond the ultimate opaque zone for gag aged 2-5 yrs; and B) qualitative amount of opaque growth observed on the edge of dorsal fin rays of gag aged 2-5 yrs collected from January-December.

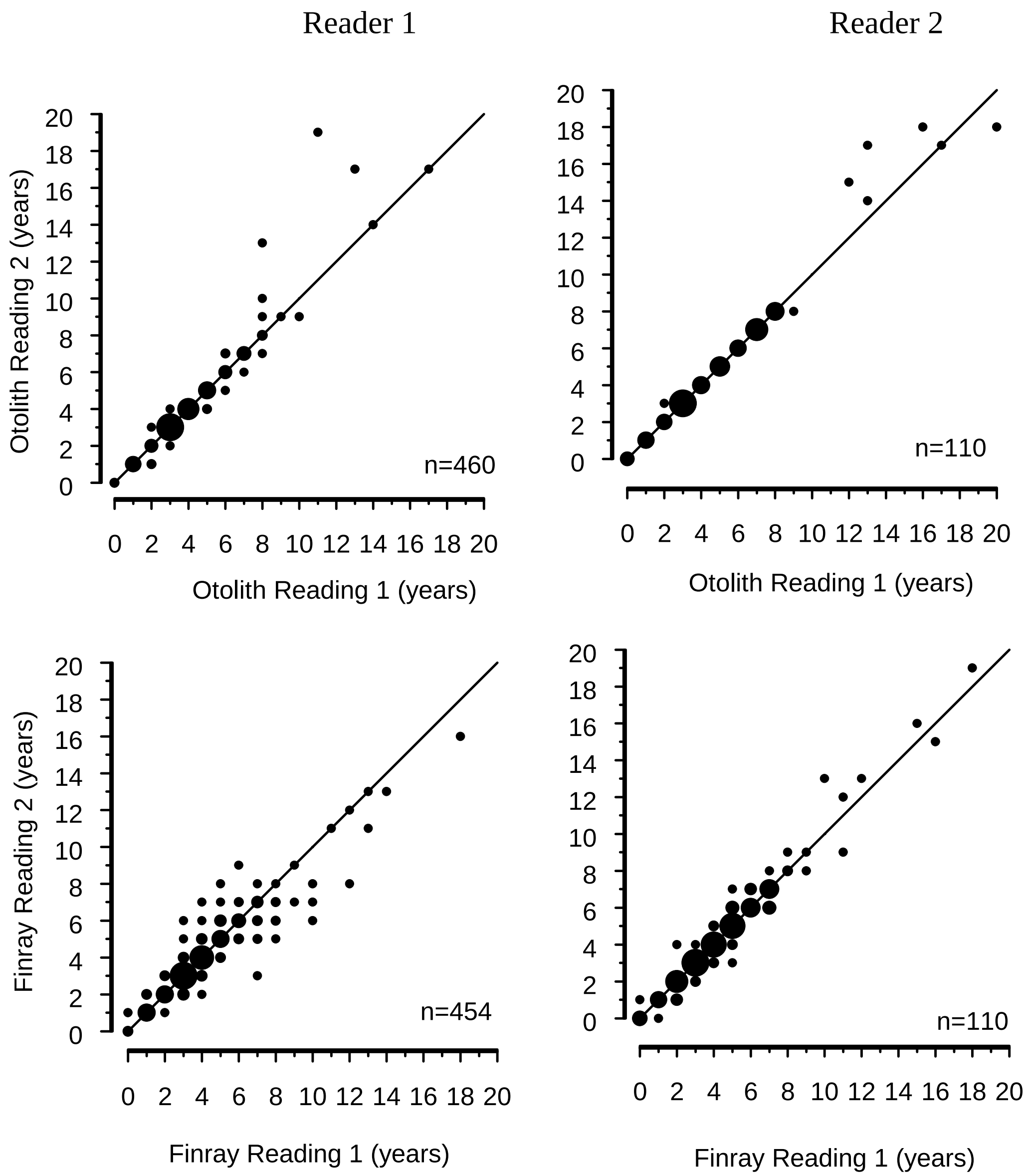


Figure 12. Scatter plot comparison of the age estimate obtained using fin rays versus otoliths for gag. The diagonal line represents comparisons where otolith ages = fin ray ages. Circle size represents the relative sample size for that age combination.

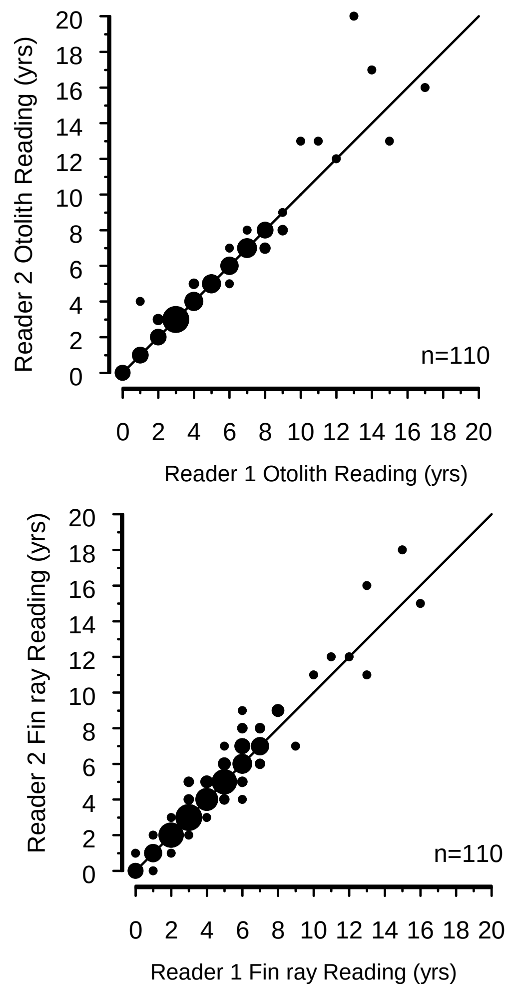


Figure 13. Scatter plot comparison of the age estimate obtained by Reader 1 versus Reader 2 using A) otoliths and B) fin rays for gag. The diagonal lines represent comparisons where age estimated by Reader 1 = age estimated by Reader 2. Circle size represents the relative sample size for that age combination.

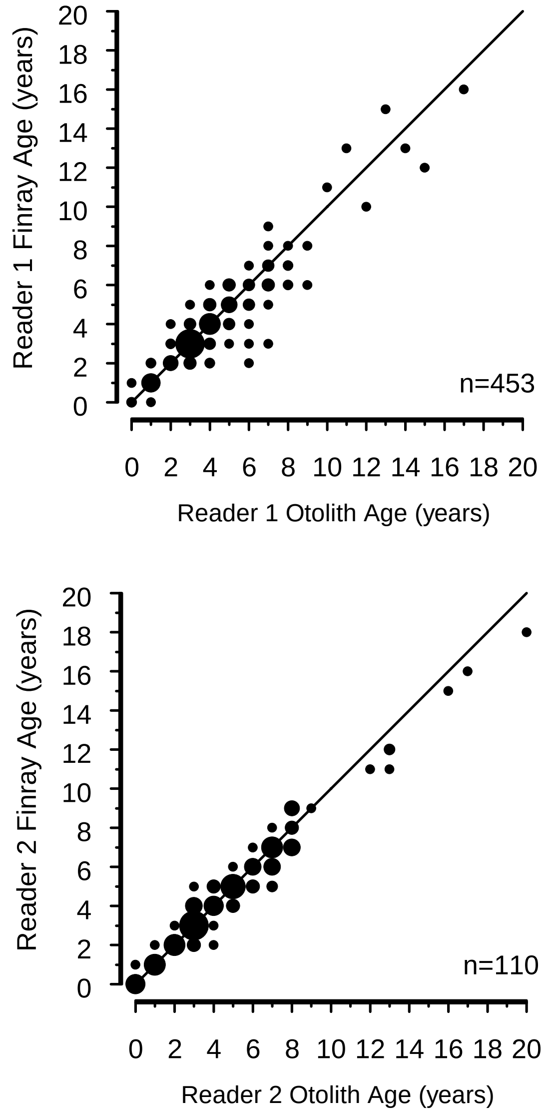


Figure 14. Scatter plot comparison of the age estimate obtained using otoliths versus fin rays for A) Reader 1 and B) Reader 2. The diagonal lines represent comparisons where age estimated for otoliths = age estimated for fin ray. Circle size represents the relative sample size for that age combination.

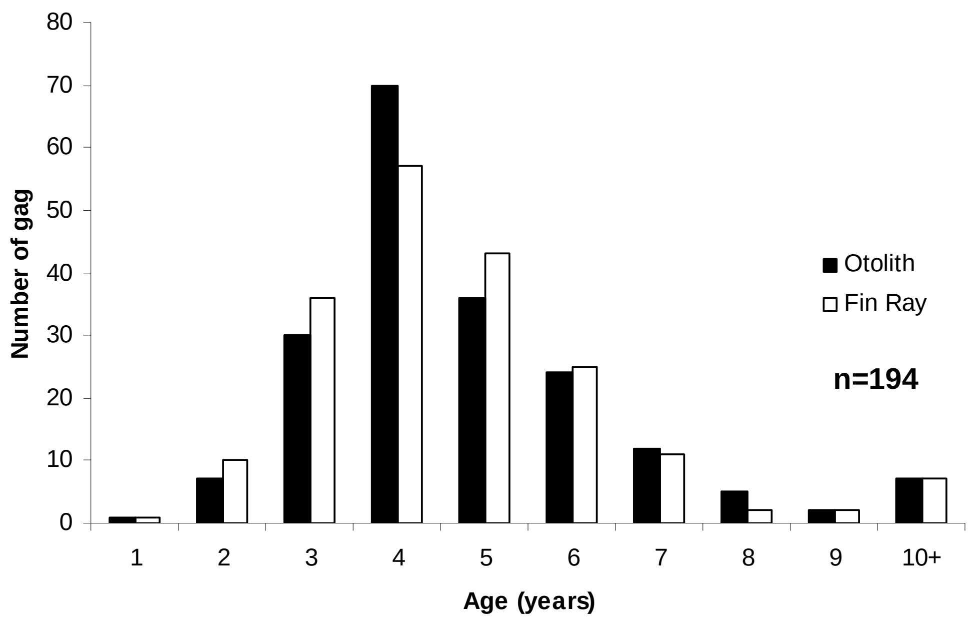


Figure 15. Derived age structure for gag grouper from otolith-assigned ages and fin ray-assigned ages. The number of samples in otolith age classes of 1 to 10+ were chosen according to the proportions of the age structure given in Fitzhugh et al. (2003).

CHAPTER 4
DISCUSSION

Aging Gag Using Dorsal Fin-Rays

Dorsal fin rays proved to be a viable, alternative nonlethal aging method for gag. Although measures of precision (i.e., aging reliability) were lower when aging gag using fin rays compared to sectioned otoliths for both within-reader and between-reader aging (Table 1), this precision was influenced primarily by either underaging or overaging gag fin rays by one year. In particular, tests of symmetry demonstrated that specific age classes of gag are prone to aging bias, which was attributed to the presence of strong false annuli or "checks" in these ages.

Ricker (1975) suggests that 80-90% agreement between sets of ages (within-reader precision) is good. Within-reader precision was consistent for both Reader 1 and Reader 2 for both otoliths (94% and 95%, respectively) and fin rays (69% and 66%, respectively), indicating that each reader had approximately the same consistency aging the structures and similar difficulty interpreting fin rays. Another moderately long-lived marine species, bluefish (*Pomatomus saltatrix*) from the Chesapeake Bay region, had similar levels of within-reader agreement (86% and 63% for sectioned otoliths and dorsal fin spines, respectively, with ages ranging from 1-14) (Sipe and Chittenden 2002).

Although between-reader agreement for gag grouper otolith ages was good (83% ranging from 0-17 years old), the relatively high variability observed both within and between Reader 1 and Reader 2 in age estimates of gag older than 10 years using otoliths (Figure 12 and 13) was unexpected in comparison to the lower variability in age estimates of these older gag using fin rays. Otoliths of older gag were difficult to age consistently due to the presence of a very wide translucent zone on the edge of the structure, with obviously either missing or indistinct opaque zones within the translucent zone. Fin rays for these same fish still had distinct opaque and translucent zones, making it easier for both readers to enumerate fin rays of older gag when compared to otoliths of older gag. Variation in otolith age estimates increasing in older gag because of the lack of clarity of the opaque zones in a wide translucent zone has also been recognized by other experienced gag otolith readers (pers. comm., Dr. Gary Fitzhugh, NMFS, Panama City). The difficulty in observing the opaque zones in older gag may occur because as the fish ages and the rate of somatic growth decreases, the proportion of opaque growth visible in the otolith is miniscule in relation to the large amount of translucent zone deposition that occurs over a longer period of reduced somatic growth.

In the present study, between-reader agreement for gag grouper otolith ages (83%) was slightly greater than between-reader agreement documented in Hood and Schlieder (1992) (76% from samples ranging from 1-17 years old). However, it is important to keep in mind that Hood and Schlieder (1992) aged more fish in older age classes (31% older than 7 years) than in the present study (5% older than 7 years). Some reef fish species are typically easier to age than gag, including white grunt with 98% agreement between readers, with a maximum age of 18 years in the Gulf of Mexico (Murie and Parkyn 2005). Johnson and Saloman (1984) also found much higher percent agreement for between-reader dorsal spine precision for gray triggerfish, at 98% (maximum age of 13 years, with most fish $\leq$ 8 years) with an APE = 0.0072.

Studies on comparative aging of fin rays or spines in relation to otoliths have met with variable results. Sikstrom (1983) noted only 49% agreement between fin rays and otoliths for arctic grayling ranging in age from 1 to 12 years, which increased to 94% ± 1 year. In Sikstrom (1983), otolith ages were generally higher than fin ray ages when they did not agree. This was attributed to difficulty in identifying the first annulus in arctic grayling and difficulty obtaining sections sufficiently close to the base of the ray (Sikstrom 1983). Agreement between structures for gag was slightly higher than agreement between structures for arctic grayling (56-62% for gag compared to 49% for arctic grayling). Although the first annulus in gag was sometimes difficult to identify, it did not cause consistent disagreement between otolith and fin ray ages. Sectioned otoliths and dorsal fin spines of bluefish also had a relatively low agreement of 52%, which increased to 68% for bluefish younger than age 5 (Sipe and Chittenden 2002). In contrast, agreement between otoliths and dorsal fin rays was 90% for white grunt less than age 10 (Murie and Parkyn 1999). Agreement over all ages (0-20 years) of gag grouper based on otoliths and dorsal fin rays appeared to be moderate (62%) in comparison to these other studies.

The ages obtained from reading otoliths in this study were assumed to be the “best” estimate of the age of the gag (i.e., 100% accurate) and were therefore useful in comparing ages obtained by other methods. This assumption is obviously a problem if it proves to be false, as proved to be the case in aging gag >10 years using otoliths. The extent to which ages of older gag based on otoliths have confounded the comparison of ages based on fin rays is presently unknown. To rectify this assumption, it will be necessary to improve the methods used currently to age older gag using otoliths, perhaps by either taking thinner sections or polishing or staining the otolith sections to increase the contrast of the opaque zones (Vanderkooy and Tisdel 2003). The acceptability of using otolith ages for these older gag as the “best” ages will depend on being able to age the fish with either equal or greater precision than the alternative aging structure (fin rays in this case).

Challenges with the Fin-Ray Method

Potential aging inaccuracies using fin rays can be largely attributed to four main problems: 1) identification of the first annulus; 2) differentiating false annuli or checks from true annuli; 3) compaction of the annuli on the edge; and 4) interpretation of the edge of the aging structure.

The first potential problem in assigning ages to fin rays of gag was identification of the first annulus. The first annulus in fin rays was sometimes partially occluded by the basal sinus that runs through the center of the ray (Figure 8A), or the region where the blood vessel sinus should be located was almost completely missing. This particular problem is not uncommon with fin ray and spine aging (Beamish and Chilton 1977). Partial occlusion and a general blurriness or lightness near the core made distinguishing the first year difficult on some rays. This problem was less common in fish aged 0-3 than in fish 4 years of age and older. Beamish and Chilton (1977) proposed measuring the mean radius of the first and second annulus as a method for determining if the first annulus has been reabsorbed or occluded by the sinus in the center of the fin ray. Penha et al. (2004) further proposed measuring the mean radius of the first annulus and comparing between age groups to determine if the first annulus has been missed in any age groups. These methods were applicable for determining if the first annulus had been missed when reading fin ray sections of gag since the diameters of the first and second annuli were significantly distinct from one another (Figure 6). In addition, there was no trend of increased first annular radius with age, which would be expected if resorption or occlusion of the first annulus was occurring in older fish. An occluded first annulus in fin rays of some species is a serious problem due to vascularization of fin rays. However, in comparison to lingcod, gag do not have heavily vascularized fin rays (pers. comm., Debra Murie). Being able to identify the approximate location of the first and second annuli in gag fin rays by using a measurement based on their diameter is advantageous for both readers lacking experience with the method and experienced readers that need to revisit the reference aging collection prior to reading additional fin rays.

Secondly, problems in differentiating checks from true annuli led to systematic aging bias in 3, 4, and/or 7 year old gag, based on tests of symmetry. The systematic bias in underaging 3 year old gag, or aging 4 year old gag as either 3 or 5 years, was most likely due to misinterpretation of checks versus annuli. Between the ages of 3 and 6, checks were particularly difficult to distinguish from annuli and gag in this age range had a tendency to have each annulus split into two or more distinct checks. Knowing this, readers may have had a preconception of the split-annulus pattern and may have overcompensated by putting 4 year old gag in age class 3 when in fact each of the observed “checks” was actually an annulus. This may also be the case for 7 year old gag that were systematically underaged by 1 year. When aging, the reader may have assumed that since gag of ages 3-6 had problematic checks, this may extend to gag with 7 apparent annuli.

The compaction of annuli at the edge of fin rays in older gag, as a potential source of underaging, was not as significant a problem as initially suspected based on other studies using fin rays (Beamish and Harvey 1969; Beamish 1981; Sipe and Chittenden 2002). If underaging of fin rays was occurring then a scatter plot of otolith versus fin-ray ages would reveal data consistently below the 1:1 line that indicates perfect agreement between structures. For fin rays, this typically occurs when the annuli become compacted on the edge of the structure because readers cannot differentiate the number of annuli at the edge, and therefore there is an increasing difference between the otolith ages and the fin ray ages with increasing fish age (Chilton and Beamish 1982). For gag, age estimates for fin rays by Reader 1 began to show evidence of underaging between age 5 and age 9, beyond which there appeared to be no systematic underaging as evidenced by data falling to either side of the 1:1 line (Figure 14A). Again, this aging error can be attributed to problematic checks in these age classes, as evidenced by systematic underaging by 1 year in age classes 3 and 7. This was different than the systematic underaging of fin rays by 1 or 2 years that was evident in gag >10 years aged by Reader 2 (Figure 14B). However, obtaining resolved ages for these older gag using otoliths was problematic for both readers (Figure 13), with Reader 1 concluding that these older gag were being underaged, which was suppported by Reader 2 consistently assigning these older gag into older age classes than Reader 1 (Figure 13). Without increased precision in aging older gag using otoliths, which is supposed to provide the “best” age for the fish, it was not possible to state that the difference from the 1:1 line was indicative of a systematic or unacceptable underaging error. The results of this study indicate that older gag can be aged more precisely with fin rays than with otoliths. This needs to be further examined with larger sample sizes.

A fourth type of aging inaccuracy can be attributed to difficulty in interpreting the edge of the fin ray. The edge on gag fin ray sections often appeared cracked, making annuli difficult to distinguish. The cracked edge may be attributed to a thin layer of skin that was not removed through the short boiling process. Although fins are boiled until the skin is soft and then lightly brushed, a thin layer of skin may remain. Even a small amount of skin folded over the edge of the sectioned fin ray can obscure the edge. Another possibility is that the outer edge of the dried or frozen fin ray is easily fractured during the drying, mounting and/or sectioning process. This damage may result in tiny cracks that are present along the outer edge of the fin. Alternative methods of removing the skin and associated tissue around the fin rays should be examined if it is found that that observed cracking results from the processing methodology.

Annulus Formation in Otoliths and Fin Rays

Understanding the timing of annulus formation is critical when comparing different aging methods, but is often overlooked or limited to only one of the aging structures being examined. In particular, timing of the deposition of annuli in paired otolith and fin ray samples has never been compared to our knowledge. This comparison is necessary in order to determine if ages estimated using different methods can be interpreted in the same manner and is essential for placement of fish into proper age classes. Qualitative marginal-increment analysis for otoliths and fin rays indicated that the two structures deposited complete opaque and translucent zones at approximately the same time each year. It is important to note that qualitative marginal-increment analysis for otoliths did not reveal a high proportion (>0.5) of opaque growth in any months. The highest proportion of gag otoliths with complete opaque zones identifiable on the edge occurred in May and June. This is most likely due to fast growth of the otolith occurring in a very short period of time, associated with a growth spurt in the fish. The same is true for opaque zones in fin rays, which represent faster zones of growth. The highest proportion of completed opaque zones occurs from June-August, but does not become greater than 0.5. This is also likely due to concentrated, short, fast growth periods. Larger sample sizes in the summer months may have produced more distinctive patterns in qualitative marginal-increment analysis. It is also possible that the variability in the start of fast growth periods, combined with the short duration of fast growth, may have made distinctive patterns (where the proportion of fish with an opaque zone on the edge is >0.5) difficult to observe. The low proportions of the opaque zone on the edge of the structures may also be a result of the applied aging criteria. Annuli were designated as "on the edge" of the otolith if the opaque zone was visible near the sulcus. However, annuli can often be identified on the dorsal and ventral tips of the otolith sections earlier than near the sulcus. If the criteria for otoliths was extended to include annuli visible on the edge of any part of the otolith section, the proportion of otoliths with annuli on the edge would likely increase. Criteria for fin rays could also change the proportion of annuli judged "on the edge" of the structure. The criteria used in this study designated annuli as "on the edge" if a translucent zone was visible on the edge of the oblique, inner part of the fin ray section, as well as on some portion of the outer edge of the fin ray. Since fin rays lay down the translucent zone on the inner, oblique area of the fin ray first, if the criteria were changed to include all fin rays with translucent zones on the oblique sections only as "on the edge", the proportion with annuli on the edge would increase.

Marginal-increment analysis provided evidence of annual deposition in the fin rays and otoliths of young (2-5 yr old) gag (Figure 11). Since the otolith aging method of gag has not been validated for fish >7 yr of age (Collins et al. 1987; Hood and Shlieder 1992), it must be assumed for now that one annulus is deposited each year in older fish as well. Whether this is correct or not is not of paramount importance for the present study because the deposition of annuli is similar in both of the aging structures under consideration when viewed relative to one another (rather than in absolute terms). So otoliths and fin rays appear to deposit annuli at the same rate in older gag, regardless of the accuracy of that rate.

Marginal-increment analysis was fundamental to understanding how to place gag into age classes. Because opaque zones were enumerated in otoliths and translucent zones were enumerated in fin rays, fish would have been placed into incorrect age classes if the timing of the deposition of these zones was not properly understood. In most studies investigating alternate aging methods for a fish, two or more aging structures are enumerated independently and then compared. Often, fish are placed into age classes based solely on annual mark counts (Crumpton et al. 1984; Sipe and Chittenden 2002; Ihde and Chittenden 2002). Having young gag (0 and 1 yr olds) in the samples allowed us to determine that although gag have an opaque core to their otolith, they do not lay down their first opaque zone (annulus) in the otolith until the second calendar year of life when they are ~15 months old. In contrast, the fin ray does lay down a translucent zone (annulus) in the first calendar year when the fish is ~8 months old. Due to the enumeration of opaque zones in otoliths and translucent zones in fin rays, these structures would place a gag into different age classes if the timing of deposition had not been evaluated (i.e., by otolith a gag would be placed in age 0 in the fall of their first year and by using the corresponding fin ray the same fish would be placed into age 1). Without understanding the timing of deposition (via edge or marginal-increment analysis) the aging structure under investigation may be interpreted inappropriately. Analyzing the timing of deposition and composition of annuli are therefore crucial steps in understanding the comparability of two aging structures.

Opaque zones observed in gag fin rays visibly represented somatic growth of the fin (i.e., there was tissue deposition). If it is assumed that the growth in the dorsal fin rays is comparable in timing to the rest of the somatic body growth of the gag, then this suggests that the opaque zone of the fin rays represents relatively fast growth in gag, whereas the translucent zone represents relatively slow or no somatic growth. Narrow translucent zones in fin rays occurred over an extended period of time in which water temperatures decreased and stayed generally cooler in coastal waters of the Gulf of Mexico where the majority of gag were collected (Kline 2004). With decreased water temperature somatic growth is slowed, and hence growth of the fin ray is minimal. Wide, opaque zones in fin rays that are believed to be associated with relatively faster somatic growth occurred as water temperature increased in the spring and early summer months.

Conventional aging methods for marine reef fishes in the southeastern U.S. enumerate opaque zones, with the understanding that these opaque zones represent a period of relatively slower growth (Hood and Schlieder 1992; Harris and Collins 2000; Vanderkooy and Tisdel 2003). However, based on the near simultaneous deposition of complete opaque and translucent zones in otoliths and fin rays of gag (Figure 11A and 11B), it would appear that the opaque zone in gag otoliths more likely represents a period of relatively faster growth. The translucent zone in gag fin rays was narrow and was observed on the edge of the fin ray structures for a relatively long period of time, from fall through to spring (Figure 11B). If fin-ray growth increments were indicative of body growth, then somatic growth of gag was at a minimum through the late fall and into the early spring. Although the translucent zone in the otoliths was also observed on the edge of the otolith over an extended period of time similar to the fin rays, the translucent zone nevertheless continued to be deposited over that time period and was therefore relatively wide. The continued deposition of the translucent zone in the otoliths of gag over a period of little or no apparent somatic growth (i.e., dorsal fin growth) indicated that otolith growth at that time was uncoupled from somatic growth in gag (Casselman 1990; Wright et al. 1997). This uncoupling of somatic growth from otolith growth has been observed previously in other fishes. Most notably, experimental studies on arctic char have shown that even once somatic growth has ceased, otoliths continue to grow over the life of the fish (Mosegaard et al. 1988). In the southeastern U.S., Murie and Parkyn (2005) have also noted that linear regressions relating white grunt TL to otolith radius result in poor regression fits ($r^{2=}$0.43-0.51), indicating that somatic growth is not directly proportional to otolith size. Thus older white grunts (e.g., 14-18 years old) have been observed to have approximately the same TL as younger fish (e.g., 4-5 yrs old), but the latter group have larger otoliths (increased otolith radii) (Murie and Parkyn 2005). Wright et al. (1997) also suggest that there is evidence against any direct functional relationship between somatic growth rate and otolith accretion rate. Experimental studies that have either reduced or eliminated food intake of fish have noted that the fish stop growing in body size and weight, yet their otoliths continue to growth, even through periods of starvation (Casselman 1990; Wright et al. 1997). If deposition of calcium carbonate and otolin is physiologically linked to metabolic rate, including maintenance metabolism throughout the year, then deposition in the otoliths during periods of little or no somatic body growth is reasonable (Wright et al. 1997). In addition, otoliths are used as a sensory structure by the fish for hearing and balance (Victor and Brothers 1982) and their increase in size may be at least partially decoupled from an increase in the size of the fish's body due to their function, as has been observed for other sensory structures such as eyes (Pankhurst and Montgomery 1994).

Implications of a Nonlethal Aging Method for Gag

In summary, this study showed that fin rays have the potential to be a useful nonlethal aging structure for gag. There are currently no critical standards for acceptable levels of APE, but Morison et al. (1998) recommends a threshold APE of 5 for production aging. In this study, within-reader APE for fin rays was 4.85-6.38 and between-structure (otoliths versus fin rays) APE was 5.94-6.21. The advantage of the fin-ray aging method is that the fish do not need to be sacrificed for age determination. Removal of dorsal fin rays, in particular, should provide minimal harm to the fish. Fin rays may be a better structure to remove from the fish than dorsal fin-spines, because dorsal fin-spines are used for defense against predators whereas fin rays largely serve as a supportive structure for the fin. Although this study focused on the use of dorsal fin rays, dorsal fin spines may also prove to be a useful structure and should be further examined.

In addition, to further reduce possible harm to the fish, the recommendation of this study is that fin rays be removed from the dorsal fin where the rays meet the body of the fish. By removing the fin rays at this level, all annuli were retained and minimal risk of infection would be imposed on the fish. It is therefore not necessary to remove the fin rays at the base of the pterygiophores, which is advantageous for groupers since they have a fleshy base to their dorsal fin and fin excision at a lower level could create an open wound.

The disadvantages of the fin-ray method are that fin rays are more time consuming to prepare and process (Murie and Parkyn 1999) and interpret than otoliths. For older fish (>8 years old), the fin ray method needs to be further evaluated to determine its reliability, but this comparison ultimately depends on obtaining reliable age estimates for these same fish using otoliths, which proved to be a significant problem that needs further resolution. Significant aging errors may be caused by using only fin rays to age fish beyond the age at which the method breaks down. This could lead to error in estimations of gag grouper population parameters, such as mortality and growth rates. For the limited gag samples >8 years old that were assessed in this study (n=7), the fin-ray method was useful and under- or over-estimated gag ages by only 1-2 years in most cases. The test of symmetry did not indicate any systematic bias for older fish and within-reader precision was higher for fin rays (when aging older fish) than for otoliths. Use of the fin ray method for aging older fish should be further investigated.

It was also apparent that developing the methodology and interpretation of using fin rays to age gag in a nonlethal manner could be of direct benefit to ongoing management efforts for gag, as well as protected Nassau and goliath groupers. For gag, the fin-ray method has important applications for studies currently being conducted offshore in the Middle Grounds of the Gulf of Mexico, where specific areas are closed to fishing during the spawning season each year to protect aggregations of gag and other reef fishes (Gulf of Mexico Fishery Management Council 2001). As part of the examination of the effectiveness of these reserves, the age structure of the gag populations inside and outside of these reserve areas are being assessed to determine if the age structure of gag in the reserve areas are showing signs of recovery (i.e., proportion of gag in older age classes is increasing) (pers. comm., Dr. Chris Koenig, Florida State University, Tallahassee, FL). Gag from the reserve areas are therefore currently being aged using the nonlethal fin ray aging method so that age samples can be taken in a non-destructive manner (i.e., removing 1-2 fin rays versus the otoliths). The fin-ray method for gag has been shown to be a viable nonlethal alternative to otolith aging and is potentially more accurate and precise than otoliths when aging fish older than 10 years old.

Using gag as a model grouper species, the fin-ray aging method has also been demonstrated to be an appropriate method to explore for use in aging other serranids, such as goliath and Nassau groupers. Like gag, these serranids are moderately long-lived, warm-temperate fish with overlapping latitudinal ranges. This would be an especially appropriate method for aging these threatened serranids since it would allow for monitoring a rebuilding of the age structure of their stocks without sacrificing a large number of already scarce fish.

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BIOGRAPHICAL SKETCH

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