# Age Validation and Growth of Gray Triggerfish, *Balistes capriscus*, In the Northern Gulf of Mexico

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# AGE VALIDATION AND GROWTH OF GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, IN THE NORTHERN GULF OF MEXICO

by

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ABSTRACT

AGE VALIDATION AND GROWTH OF GRAY TRIGGERFISH, *BALISTES CAPRISCUS*, IN THE NORTHERN GULF OF MEXICO

Carrie Lee Fioramonti

A rearing experiment was conducted in which adult gray triggerfish dorsal spines and fin rays were chemically marked with oxytetracycline. Experimental results validated that one translucent zone was deposited in spines and rays during winter/early spring; however, fin rays proved to be less reliable than spines for age estimation (n = 27). Spines collected from fish (n = 2,391) sampled in the northern Gulf of Mexico between 2003 and 2010 were sectioned and aged by two independent readers via counts of translucent zones. Marginal increment analysis verified that a single translucent zone was formed in spines during winter/early spring. A von Bertalanffy growth function was fit to the entire data set [$L_t = 521\ (e^{(0.274*(t+0.12))})$], as well as by sex, fishery, and region. Results from a three-way analysis of variance indicated no significant difference in size at age between sexes, but fishery and region effects were significant. However, a significant interaction between region and fishery effects confounded interpretation of main effects and precluded inference about regional growth differences given that differences in selectivity among region-specific predominant fisheries may have resulted in observed regional differences in size at age. Overall, study results indicate dorsal spines can be used to age gray triggerfish accurately, and that aging data may be useful to estimate growth or as inputs to age-structured stock assessment models.

# INTRODUCTION

## *Life History*

The gray triggerfish, *Balistes capriscus*, is a moderately long-lived member of the Gulf of Mexico (GOM) reef fish community. Gray triggerfish inhabit tropical and temperate waters between depths of 12 and 42 m, and the species ranges from Norway to the northwestern coast of Africa in the eastern Atlantic Ocean (Ofori-Danson 1989) and from Nova Scotia to Argentina in the western Atlantic, including waters of the GOM and off Bermuda (Harper and McClellan 1997). Maximum reported size is 725 mm fork length (FL) for male gray triggerfish and 561 mm for females, while maximum observed longevity is 14 years for males and is 12 years for females (Johnson and Saloman 1984; Hood and Johnson 1997).

Gray triggerfish become reproductively mature between ages 1 and 3 years, with spawning in the GOM beginning in May, peaking in June and July, and decreasing in August (Ingram 2001). Males construct several demersal nests and perform elaborate courtship behaviors (e.g., encircling females and coloration changes), attracting a harem of females with which to mate (Makichan and Szedlmayer 2007). Once fertilization occurs, males attentively guard the territory surrounding nests and females guard their eggs until hatching, which typically occurs within 48 hours (Ofori-Danson 1990; Bernardes and Dias 2001; Makichan and Szedlmayer 2007). It is notable that during the spawning season both males and females appear to exhibit limited feeding due to their

territoriality and resistance to capture by baited hooks or traps (Ingram 2001; Makichan and Szedlmayer 2007).

Planktonic larvae and juveniles up to 175 mm standard length (SL) are associated with *Sargassum* communities (Casazza and Ross 2008; Wells and Rooker 2004). Adults and juveniles greater than 160 mm SL are commonly associated with coral reefs, rocky outcroppings/hardbottom, and wrecks, and they are often the earliest successful colonizers of artificial reefs (Vose and Nelson 1994). Adults are diurnal feeders and their dentition enables them to prey upon both armored and non-armored invertebrate prey items, such as echinoderms, mollusks, and crustaceans, that are associated with both sandy bottom and reef habitat (Frazer et al. 1991).

*Gulf of Mexico Gray Triggerfish Fishery*

Until recently, gray triggerfish were not heavily targeted or considered an important food resource in the reef fish fishery. However, gray triggerfish have become an increasingly targeted species both commercially and recreationally due to increased regulations on other reef fishes, such as snappers and groupers (Valle et al. 2001; Bernardes 2002). Consequently, landings data for triggerfish species increased substantially beginning in the mid-1980s, followed by a significant decline from the mid-1990s to present (Southeast Data Assessment and Review [SEDAR] 2006, 2012; Figures 1 and 2). It is important to note that gray triggerfish commercial landings data prior to 1993 incorporate all species within the triggerfish family. It was not until 1993 that protocols to identify each species were introduced and are still lacking for data reported from Florida state waters.

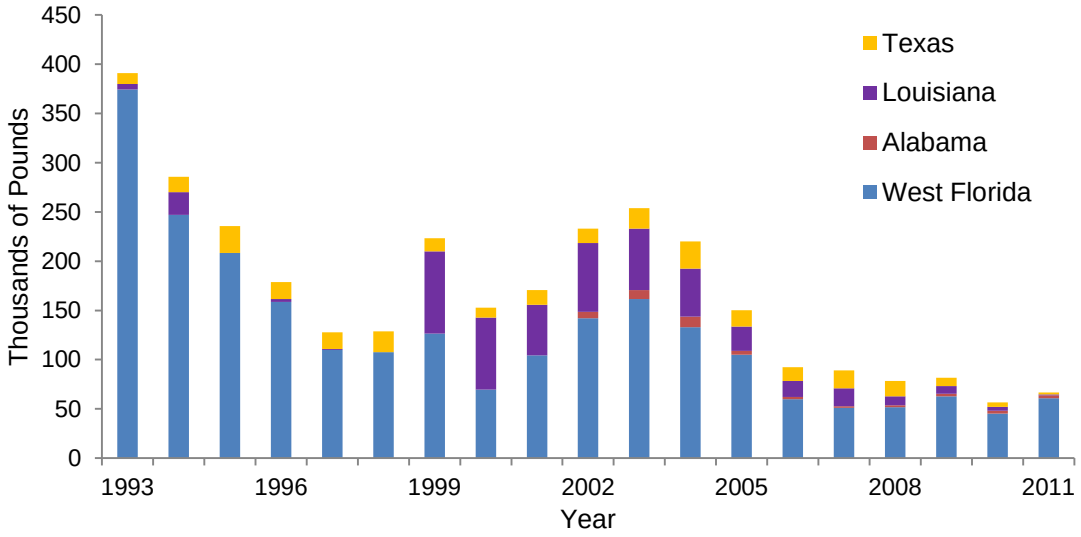


*Figure 1*—Estimated U.S. commercial gray triggerfish landings in the Gulf of Mexico (GOM) by state from 1993 through 2011. Florida estimates include all triggerfish species. Data source: National Marine Fisheries Service (NMFS), Fisheries Statistics Division, Miami, FL.

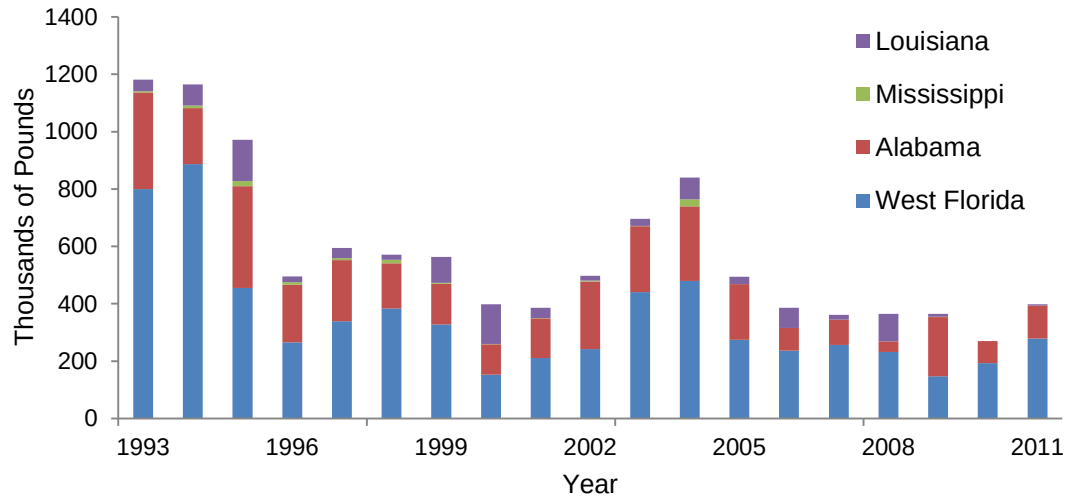


*Figure 2*—Estimated U.S. recreational gray triggerfish landings in the Gulf of Mexico (GOM) by state from 1993 through 2011. Data source: National Marine Fisheries Service (NMFS), Fisheries Statistics Division, Silver Spring, MD.

The National Marine Fisheries Service's (NMFS) early attempts to estimate GOM gray triggerfish stock status involved surplus production modeling (Goodyear and Thompson 1993; Harper and McClellan 1997; Valle et al. 2001). More recently, age-structure modeling was attempted during a SEDAR benchmark assessment for the stock (SEDAR 2006). However, due to limitations of the age structure data, two different types of assessment models were employed to estimate stock status of gray triggerfish: a non-age-based aggregated stock production model and an age-based stock production model.

Age-based models typically are preferred over models that do not incorporate age structure because they enable life history parameters, such as age at maturity, age at recruitment, and growth rates, to be modeled as part of an assessment (Haddon 2001). However, aging techniques must be validated or verified (Campana 2001), which has not been sufficiently performed for gray triggerfish. Marginal increment analysis, maximum likelihood estimation of size at age, and spine radius-FL regression (verification) have been the only methods implemented (Ingram 2001; Moore 2001), and sample sizes have been limited.

Gray triggerfish were first included in the Gulf of Mexico Fishery Management Council's (GMFMC) Reef Fish Fishery Management Plan (RFFMP) (GMFMC 1981) in 1989 as an addition to the list of species in the plan's Amendment 1 (GMFMC 1990), with the primary goal being to protect their spawning stock biomass. Amendment 12 to the RFFMP (GMFMC 1995) established an aggregate recreational daily bag (possession) limit in federal waters for all reef fish species, including gray triggerfish, but no individual bag limit for gray triggerfish. Amendment 16b (GMFMC 1999) introduced a minimum size limit of 12 inches FL for gray triggerfish. Amendment 30A (GMFMC

2008) increased the size limit from 12 to 14 inches FL with the goal to end overfishing and rebuild the GOM stock, which was in response to estimates resulting from the 2005 benchmark stock assessment of gray triggerfish that the stock was overfished and undergoing overfishing (SEDAR 2006). The goal of this regulation was to decrease total landings by 60% and improve the likelihood of stock recovery (GMFMC 2008).

Previous stock assessments had data limitations due to insufficient age data, particularly with regards to very young and very old fish, as well as restricted locations from which samples were collected. Therefore, convergence of growth models was an issue as was any form of spatial comparison of growth. Therefore, sampling efforts in this study were focused on obtaining data that were lacking in previous aging studies, such as fishery-independent trawl samples of juveniles, samples of large, old fish captured in various fisheries, and a regional representation of fish captured in the north central GOM (NCG) and from the eastern GOM (EG) along the west Florida shelf.

*Aging Techniques*

Fish age is most often determined using hard-parts such as otoliths (Casselman 1990), scales (Erickson 1983), dorsal fin spines (Beamish and McFarlane 1985; Lessa and Duarte-Neto 2004), fin rays (Beamish and Fournier 1981; Murie et al. 2008), vertebrae (Alves et al. 2002), or cleithra (Babaluk and Craig 1990; Casselman 1990). Sagittal otoliths tend to be the preferred structure to age most bony fishes as they are inert once formed, thus alternate opaque and translucent zones are preserved in their structure (Wright 1991). Unfortunately, gray triggerfish otoliths are difficult to locate and extract due to their small size (<2 mm), and no ageing protocols are available due to their size and shape (Bernardes 2002; Moore 2001). Therefore, other aging structures such as

spines, fin rays, opercula, or vertebrae must be considered for aging gray triggerfish. A key assumption is that the structure being used has a pattern of opaque and translucent zones that can be interpreted accurately as annuli.

Several authors have used the first dorsal spine to age gray triggerfish (Johnson and Saloman 1984; Ofori-Danson 1989; Ingram 2001; Moore 2001; Bernardes 2002). Age determination is accomplished by identification of translucent zones within sectioned spines that are presumed to be formed annually. The rate of opaque/translucent zone formation varies with fish growth, leading to distinct zones and patterns within a spine section. Zones representing faster growth are relatively wide within sections and opaque under transmitted light, and zones corresponding to slow growth periods are narrow and appear translucent (Lessa and Duarte-Neto 2004). Periods of slow growth appear to correspond to times of lower temperatures and food availability during the winter months and are evident in the more compacted matrix of the bone which causes the translucent and narrower zone; the converse is true of the opaque zone (Ingram 2001). This is exactly opposite to the pattern observed in otoliths where zones of fast growth in otolith sections are translucent and slow growth zones are opaque (Jearld 1983).

Translucent zones (i.e., slow growth zones) are concentric rings in a spine cross section that may or may not correspond to a period of annual growth. This can only be determined through validation of the periodicity of translucent zone formation. Ingram (2001) concluded from relative marginal increment analysis results that translucent zones in gray triggerfish dorsal spines are formed during the winter months from December to early February, but also may form during the summer spawning season. It can be difficult to discern whether a given translucent zone was formed during winter or whether it is a

spawning check. Ingram (2001) hypothesized that the appearance of “doublets” (two translucent zones formed close together) in spines was due to winter deposition of a translucent zone caused by lower metabolism followed by temporary fasting of spawning/nesting triggerfish during summer. However, annual translucent zone formation in gray triggerfish spines has not yet been validated, a process in which spines are chemically marked and then fish examined later to test if one translucent zone formed per year. Validation of aging structures as forming annual growth zones is critical to ensure correct estimation of growth rates or for estimating catch at age used in stock assessments to estimate production and potential fishery yield (Beamish and McFarlane 1983).

The preferred and most reliable method for age validation is the mark-recapture of chemically tagged fish, ideally across all age classes (Beamish and McFarlane 1983; Campana 2001). Validation is considered most accurate with tagged individuals in the natural environment. It is achieved by marking tagged fish of all size and age classes with calcium-binding compounds, such as oxytetracycline (OTC), calcein, or alizarin, recapturing them at a later date, and then observing their hard parts for subsequent growth following absorption of the chemical mark. If opaque and/or translucent zones are observed distal to the chemical mark in hard parts prepared from recaptured fish, then those zones were deposited during the time the fish was free, thus the periodicity of zones can be discerned. This method has been applied to a number of species and aging structures, such as goliath grouper (*Epinephelus itajara*) otoliths (Bullock and Murphy 1992), goliath grouper spines (Brusher and Schull 2009), spiny dogfish (*Squalus acanthius*) second dorsal spines (Beamish and McFarlane 1985), lingcod (*Ophiodon*

*elongates*) fin rays (Cass and Beamish 1983), pike (*Esox lucius*) cleithra and fin rays (Babaluk and Craig 1990), round sting ray (*Urobatis halleri*) vertebrae (Hale and Lowe 2008) and northern pike otoliths, scales, and cleithra (Casselman 1990). Hood and Johnson (1997) attempted to validate the periodicity of translucent zone formation in the first dorsal spine of gray triggerfish by OTC marking in an indoor aquaculture facility with constant light and temperature. Spine sections of those fish did not show any zone deposition after the OTC marks. However, the deviation from natural light and temperature fluctuations may have altered normal physiological processes and explain the absence of growth zones in spines. Outdoor enclosures or tanks may be a better approach to replicating natural conditions, particularly light cycles (Natanson 1993; Campana 2001).

Validation of absolute age differs from verification in that it involves examining the periodicity of opaque and translucent zone formation in hard parts of known-age fish or in fish of all age classes of interest (Beamish and McFarlane 1983; Campana 2001). Verification is a term that describes corroboration of age estimates through indirect methods, such as marginal increment analysis and spine radius-FL regression. Another verification method is hard part comparison, in which two different aging structures are used for age determination and ages derived from them compared for corroboration. Fin rays are structures sometimes used for this purpose. Early work suggesting fin rays may be valid structures for aging fish species was conducted by Beamish (1981) and Chilton and Bilton (1986) to determine ages of walleye Pollock (*Theragra chalcogramma*), pacific cod (*Gadus macrocephalus*), albacore (*Thunnus alalunga*) and Chinook salmon (*Onchorhynchus tshawytscha*). Fin rays of temperate fish tend to display more distinct

banding versus those of semi-tropical to tropical fish, such as white grunt (*Haemulon plumier*) (Murie and Parkyn 2005), goliath grouper (Bullock and Murphy 1992; Murie et al. 2008; Brusher and Schull 2009), and gag (*Mycteroperca microepis*) (Debicella 2005; Murie et al. 2008). Gulf of Mexico gray triggerfish fall into the latter category of being a semi-tropical to tropical fish, thus likely making interpretation of fin rays difficult. Authors of most comparison studies compared the fin ray age determination to validated ages from otoliths. This poses a special concern in regard to gray triggerfish, since age validation for the preferred hard part, the first dorsal spine, is lacking.

Comparison of opaque zones in fin rays to those of other calcified structures may be a useful method for verification purposes, but fin rays also may provide a non-lethal method for age determination once validated (Beamish 1981; Debicella 2005; Koch and Quist 2007; Murie et al. 2008). One difficulty associated with using fin rays for age determination is the ability to extract the ray immediately proximal to the pterygiophore, as opaque zones are most evident at the base of the ray (Beamish 1981; Debicella 2005; Koch and Quist 2007; Murie and Parkyn 2005; Murie et al. 2008). This should not be an issue for preliminary examination of fin rays for use in age determination for gray triggerfish as fish typically are dead prior to sampling, thus the entire dorsal fin could be removed. However, if a reliable aging product requires invasive extraction techniques, it may preclude the use of fin rays as a non-lethal aging structure.

*Variation in Life History Parameters*

Gray triggerfish are currently managed and assessed as a single unit stock in the US GOM (Valle et al. 2001; SEDAR 2006). Life history parameters such as growth, mortality, and recruitment are important parameters in fisheries assessment, and spatial

variability in these parameters has important implications for assessing population productivity (Fischer et al. 2004; Allman 2007). Differences in these parameters also can significantly affect fisheries management effectiveness; thus, it is necessary to discern whether or not separate stocks exist or there is significant spatial variation in life history parameters (Gust 2004).

Small-scale and regional variation in life history parameters of other GOM reef fishes has been observed (Fischer et al. 2004; Allman 2007; Lombardi-Carlson et al. 2008). Results from previous aging studies indicate gray triggerfish size at age appears to be highly variable (Hood and Johnson 1997; Ingram 2001; Bernardes 2002). Ingram (2001) reported growth differences on small spatial scales (10s of km) and suggested that the high site fidelity of gray triggerfish provides evidence that localized differences in age, growth, and mortality are due to environmental conditions such as resource availability and fishing pressure. In addition, ecological processes and environmental factors such as density-dependence and habitat structure may vary among reefs, as well as from region to region, thus explaining differences in demography and life history parameters (Ray and Hasting 1996; Lindberg et al. 2006; Lombardi-Carlson et al. 2008).

The goals of this study were to provide age validation and verification for the first dorsal spines, as well as to examine differences in life history parameters of gray triggerfish between the NCG and EG along the west Florida shelf. The specific objectives were to 1) validate spines and fin rays as aging structures for gray triggerfish by rearing OTC marked fish, 2) compare spine and fin ray based ages as a method of age verification and non-lethal age determination, 3) characterize growth of gray triggerfish on a GOM-wide scale and by sex, fishery, and region by fitting a von Bertalanffy growth

function (VBGF) to the spine-based age data, and 4) test if there are differences in gray triggerfish size at age between sexes, fisheries, and regions.

## OBJECTIVES AND HYPOTHESES TO BE TESTED

Objective 1: The first objective of this study is to validate aging of dorsal spines and fin rays using OTC marked fish.

$H_{0\text{-}1}$: There is no definitive pattern of opaque or translucent zones observed after the OTC mark on a dorsal spine.

$H_{a\text{-}1}$: There is a definitive pattern of zones observed after the OTC mark on dorsal spines that implies one opaque and one translucent zone are deposited per year.

$H_{0\text{-}1}$: There is no definitive pattern of zones observed after the OTC mark on fin rays.

$H_{a\text{-}1}$: There is a definitive pattern of zones observed after the OTC mark on fin rays that implies one opaque and one translucent zone are deposited per year.

Objective 2: The second objective of this study is to verify aging via hard part comparison (fin ray versus spine).

$H_{0\text{-}3}$: There is no relationship between the deposition of opaque and translucent zones found on pectoral/dorsal fin rays and dorsal spines.

$H_{a\text{-}3}$: There is a 1:1 relationship between deposition of opaque and translucent zones found on fin rays versus spines, thus implying rays could be employed as a non-lethal aging structure in future studies of gray triggerfish life history.

Objective 3: The third objective of this study is to characterize growth of gray triggerfish on a Gulf-wide scale, as well as to test for difference in size at age among sexes, fisheries, and regions.

$H_{0\text{-}4}$: There is no difference in size at age between sexes.

$H_{a\text{-}4}$: Males and females grow at different rates, thus sexual dimorphism exists and must be accounted for in fishery management.

$H_{0\text{-}5}$: There is no difference in size at age among fisheries.

$H_{a\text{-}5}$ Significant differences among fisheries implies selectivity differences may exist among fisheries that must be accounted for when examining growth rates.

$H_{0\text{-}6}$: There is no difference in size at age among regions.

$H_{a\text{-}6}$: Significant differences among regions implies differences in population dynamics and likely population structure exist among regions.

# METHODS

## *Age Validation and Verification*

Gray triggerfish were caught in October 2009 with fish traps off the coast of Panama City, Florida and transported to the NFMS laboratory in Panama City. Fish (n = 4) were held in 600 gallon aquaculture tanks with a recirculating biofiltration system from October 2009 to July 2010. The tanks were housed in buildings constructed with a translucent, vinyl covering that allowed natural light to penetrate. Each fish was tagged with a Floy FM-95 stainless steel internal-anchor tag and chemically marked by injecting with 50 mg of OTC per kg body mass. Fish were exposed to ambient light and diurnal rhythms. Water temperature was maintained with heaters during the winter months to mimic mean bottom temperature of the GOM. Salinity was monitored and maintained at approximate GOM concentration of 32-34 psu. Fish were fed cut squid, shrimp, or fish every other day throughout their captivity. At the end of the experiment, fish were euthanized in an ice-water slurry and immediately covered in foil and frozen in a dark container. First dorsal spines, dorsal fin rays, and pectoral fin rays were extracted and processed in a darkened room to prevent degradation of the OTC mark due to light exposure.

First dorsal spines were extracted by inserting a knife just posterior to a spine and cutting medially approximately 2.5 cm into the fish. Another identical cut anterior to the spine effectively cut out a “notch” of flesh that included the entire condyle of the dorsal

spine. Dorsal and pectoral fin rays were extracted by cutting below the pterygiophores of each fin using a sharp knife or scalpel. Spines and fin rays were immediately wrapped in foil, placed into zipper seal bags, and placed inside a sealed cardboard box to prevent light exposure prior to freezing.

Dorsal spines were prepared for sectioning by boiling them in water for 1 min to remove associated tissue and then also scraping the posterior groove free of tissue. Each spine was hot-glued to cardstock and 3 transverse sections (0.5-0.8 mm thickness) were cut simultaneously with four 10-cm diamond encrusted blades on an Isomet low-speed saw at 300 rpm and with 50-75g of weight on the saw's arm. Prepared sections were fixed to microscope slides with Cytoseal mounting medium (Electron Microscopy Sciences).

Fin rays were cleaned of tissue by submerging the basal portion rays in boiling water for up to 20 seconds. Tissue was gently removed from fin rays with forceps and a small, soft bristled brush and then laid flat to dry. Once dry, fin rays were embedded in Hysol 0151 (Loc-tite Corp) epoxy for sectioning.

After approximately 3 days, embedded fin rays were cured enough to section using a single, 5-cm blade on an Isomet saw at 300 rpm and with 25-50 g of weight on the saw's arm. Each fin ray was sectioned to between 0.5-0.8 mm, with 0.6-0.7 mm being the optimal thickness for reading. Section preparation was complete once a characteristic "widow's peak" was observed around the focus of the fin ray (Figure 3). Sections were mounted onto glass microscopic slides using Flo-Texx and allowed to dry.

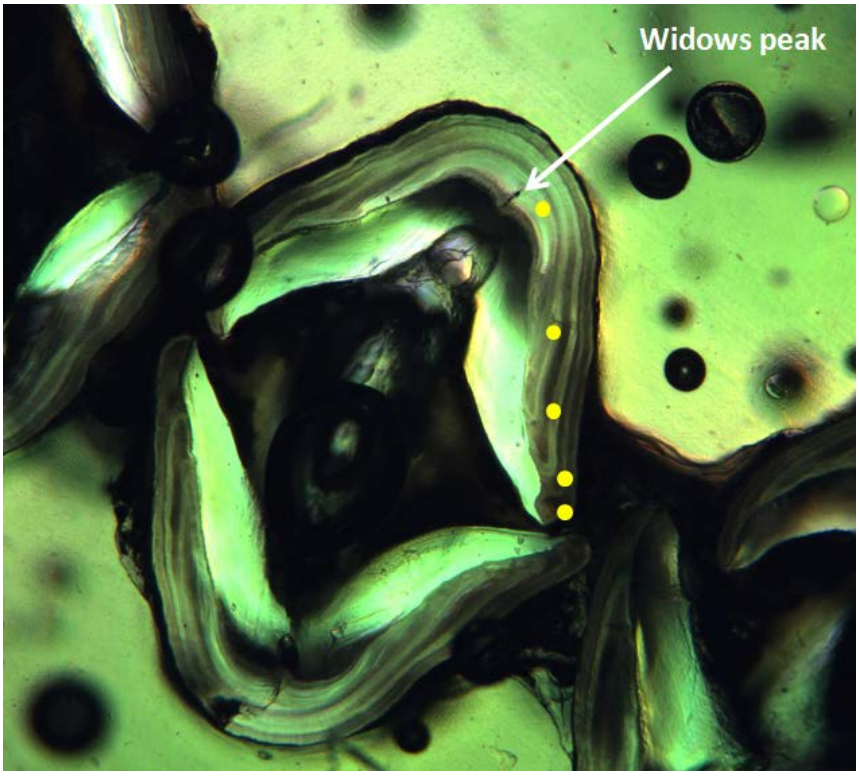


*Figure 3*.—Characteristic “widow’s peak” on a fin ray section from a 303 mm fork length (FL) gray triggerfish caught off the coast of Panama City, Florida and estimated to be 5 years old. Translucent zones are denoted with yellow circles on image.

First dorsal spines of gray triggerfish were aged by counting the number of translucent zones in transverse sections of spines observed with a dissecting microscope under 10-20x magnification and transmitted light. The margin of a spine was assigned either a “1” to indicate the presence of translucent zone on the edge or a “2” to indicate an opaque zone on the edge. Readability codes were assigned as well, to indicate ease of age determination: G = good, R = readable/fair, D = difficult, P = unreadable due to poor processing and U = unreadable due to factors other than processing.

Fin rays were aged by examining transverse sections and counting the number of translucent zones with a compound microscope under 100x magnification using transmitted light with a green filter. Fin ray margins were not assigned a margin or

readability code. Aging of fin rays was conducted separately from spines to prevent aging bias.

Oxytetracycline-marked dorsal spine sections were examined at NMFS in a darkened room with a Meiji stereo-dissecting light microscope under reflected UV light. Digital images of dorsal spine sections were captured with an ImagePro image analysis system. Fin ray sections were examined at University of Florida using a Nikon compound light microscope using transmitted UV light produced by a 100 Watt mercury-vapor bulb. Digital images of fin rays were captured with a Ken-a-vision MVP imaging system.

*Sample Collection and Aging*

Gray triggerfish samples were collected by NMFS personnel throughout the NCG and EG between 2003 and 2010. Samples were grouped into two geographic regions: NCG (NMFS statistical grids 8-11) and EG (NMFS statistical grids 1-7), with the boundary for the two sections being latitude 85° west (Figure 4).

Translucent zones were counted in first dorsal spine sections following the sectioning and aging protocols described above. Separate readings of each spine section were made by two readers (primary and secondary). Between reader precision was estimated by computing average percent error (APE) between integer ages assigned by readers using the method of Beamish and Fournier (1981) :

$$\frac{1}{N} \sum_{j=1}^{N} \left[ \frac{1}{R} \sum_{i=1}^{R} \frac{Xij - Xj}{Xj} \right] ,$$

where $N$ = number of samples ages, $R$ = number of times fish was aged, $X_{ij}$ is the $i$th age determination of the $j$th fish, and $X_j$ is the average age calculated for the $j$th fish. Any disagreement in ages was resolved by reader consensus. If a consensus could not be reached, the spine was rejected.

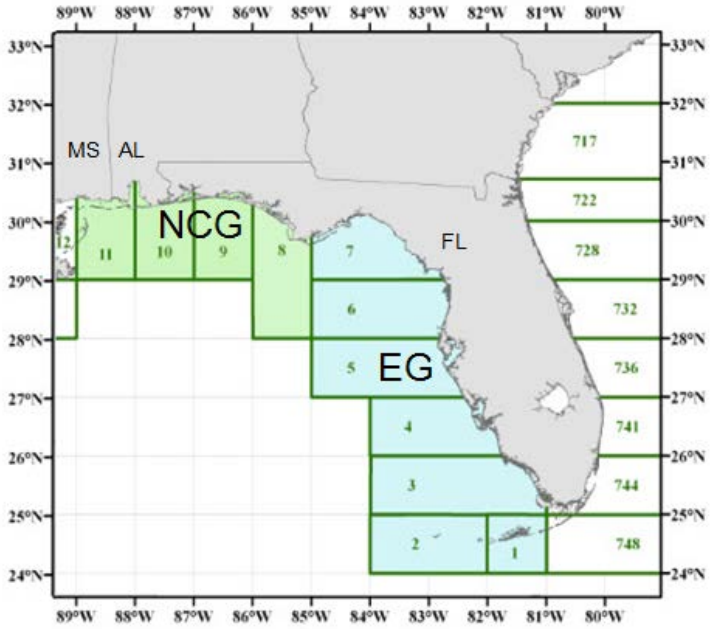


*Figure 4*.—Map of northern Gulf of Mexico (GOM) indicating regions where gray triggerfish were sampled: north central Gulf (NCG) and eastern Gulf (EG). Polygons on map indicate National Marine Fisheries Service (NMFS) statistical grids.

A relative marginal increment analysis was conducted for age-3 and age-4 fish with the method of Ehrhardt (1992). Measurements of spine radius and distance of last translucent zone to margin from fish with "fair to good" readability were used to plot the monthly average relative marginal increment. Relative marginal increment was calculated as the distance from the distal edge of the last translucent zone to the edge of a spine divided by the radius of a spine. Mean relative marginal increment then was plotted for age-3 and age-4 fish separately by month.

Fractional age was calculated from the consensus translucent zone count, timing of translucent zone deposition, assumed birth date and capture date. July 1 was selected as the mean birth date based on gray triggerfish gonadosomatic index values (Ingram

2001), and translucent zone formation was assumed to begin January 1 (see below). Therefore, at the time of translucent zone deposition the fish is only 0.5 years old. In order to adjust for this discrepancy, first one translucent zone was subtracted from the total number of translucent zones and multiplied by 365. To account for the first 0.5 year of life, 182 days was added to that product. Finally, the number of days from the beginning of the year to the capture date was added to account for the number of days the fish was alive. Fish age was then converted from days to years by dividing by 365.

*Hard-part Comparison*

Dorsal spines and matching fin rays sampled for hard-part comparison were primarily sampled from fishery-independent sources (NMFS Panama City Laboratory, NMFS Pascagoula Laboratory, Florida Wildlife Research Institute (FWRI), Gulf Coast Research Laboratory (GCRL)) and some from fishery-dependent sources (NMFS port agents, Albert LeForte and Lew Bullock). This sampling effort included a wide range of fork lengths, gears, and locations throughout the GOM. All dorsal spines and matching fin rays were stored in zipper sealed bags and frozen until processing. For fish with a fin ray sample, first dorsal spines and fin rays were extracted and processed in the same manner as described above. A t-score test was employed to test the fitted slope of the linear regression (SAS 9.2) between spine age versus fin ray age against a slope of one and a y-intercept of zero (1:1 line of agreement).

*Growth Estimation and Variation in Size at Age*

Growth was estimated for spine-based gray triggerfish size at age data and size at estimated age for juvenile data provided by Chris Stafford of FWRI (n = 198) and Jim Franks of GCRL (n = 876). The FWRI data resulted from fish captured in fishery-

independent trawl samples, while the GCRL samples came from neuston plankton net tows. Integer age first was estimated for these samples based on a bimodal pattern observed in their FL distribution (Figure 5). Integer age was assigned as age-0 for fish <100 mm FL and as age-1 for fish 100-200 mm FL. Fractional age was then assigned as described above.

Von Bertalanffy growth functions were fit to all the size at age data, size at age data without the juvenile samples, and by sex, fishery, and region, with Statistical Analysis System (SAS 9.2) (von Bertalanffy 1938):

$$L_t = L_\infty (1 - e^{(k*(t - t_0))})$$

where $L_t$ = length at time of capture, $L_\infty$ = length asymptote, $k$ = Brody's growth coefficient, $t_0$ = hypothetic age at which fish length was zero. The VBGF for sexes included the additional juvenile data as that data was collected within both regions and with all gear types, but not included for fishery and region models. Sex ratios were determined overall and for each study area, and a chi-square goodness of fit tested the resultant overall ratio against a ratio of 1:1. Lastly, differences in mean size at age for age classes 2-8 were tested with a three-way analysis of variance (ANOVA) computed in SAS with main effects of sex, fishery, and region.

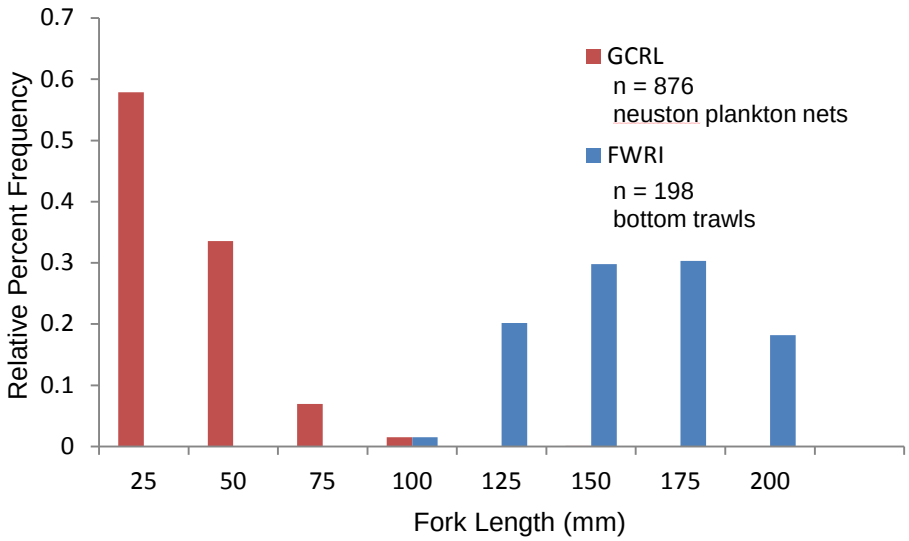


*Figure 5*.—Relative length frequency distribution for Gulf of Mexico (GOM) gray triggerfish for data supplied by Florida Wildlife Research Institute (FWRI) and Gulf Coast Research Laboratory (GCRL) showing bimodal distribution of presumed age-0 fish (0-99mm) and age-1 fish (100-200mm). FWRI data sampled by trawl and GCRL data sampled with neuston plankton nets.

# RESULTS

## *Age Validation and Verification*

Mean monthly water temperatures for aquaculture tanks were relatively consistent with bottom temperatures of GOM at depth and vicinity of capture location of reared fish (Figure 6).

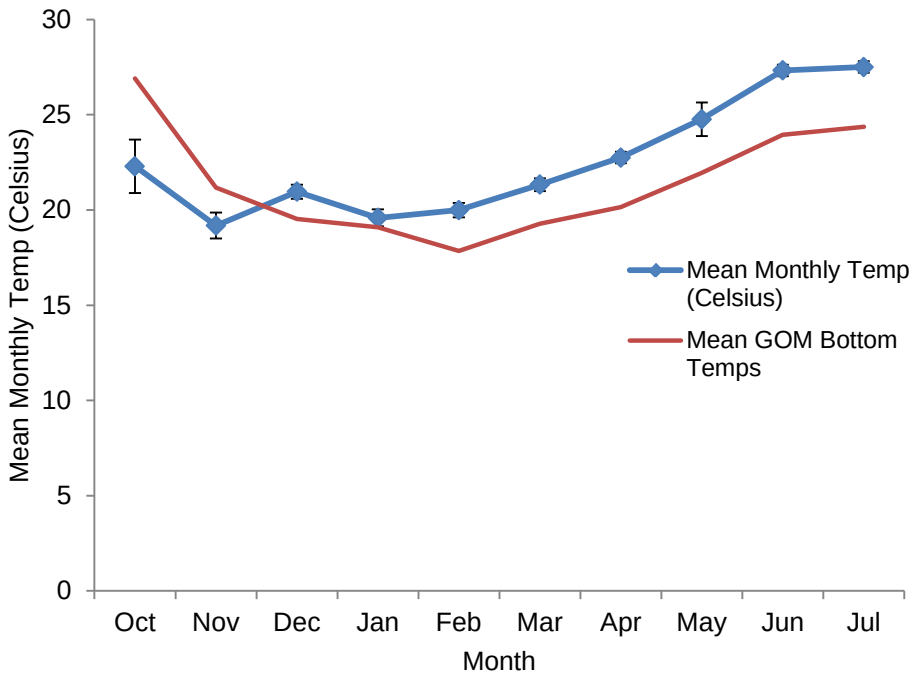


*Figure 6.*—Mean monthly water temperatures for aquaculture tanks (October 2009 through July 2010) containing captive Gulf of Mexico (GOM) gray triggerfish (blue line; error bars are standard error), and mean monthly water temperatures for northern GOM reefs at comparable depths and vicinity of capture location of captive reared fish (red line).

Results of the OTC marking experiment indicate that one translucent zone formed after the OTC mark on dorsal spines (Figure 7; A, B) and one translucent zone formed after OTC mark on fin rays (Figure 7; C, D) during winter months.

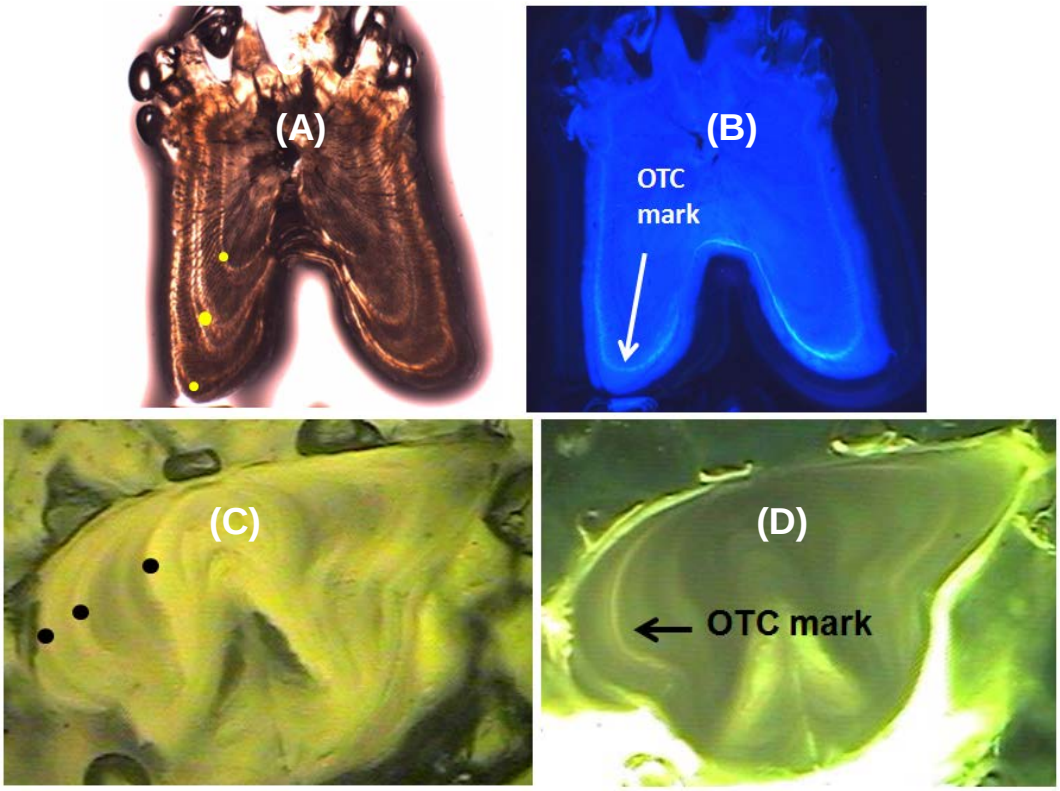


*Figure 7*.—Digital images of dorsal spine section and pectoral fin ray section from a 270 mm fork length (FL) age-3 Gulf of Mexico (GOM) gray triggerfish fish at 20x and 100x magnification, respectively. The top left image (A) is the spine section viewed under transmitted light; translucent zones are denoted with bullets. The top right image (B) is the spine section viewed under reflected ultraviolet (UV) light, which makes its oxytetracycline (OTC) mark clearly visible. The bottom left image (C) is the ray section viewed under transmitted green light: translucent zones are denoted with bullets. The bottom right image (D) is the ray section viewed under transmitted UV light showing OTC mark.

*Sample Collection and Aging*

A total of 2,391 gray triggerfish dorsal spines was collected from fishery-dependent and -independent sources during 2003 to 2010 from all GOM states in order to generate a data set with the widest range of lengths and ages possible. Sixty-two percent of samples were obtained from fishery-dependent sources (n = 1,493), with recreational samples (n = 965) outnumbering commercial samples (n = 528). The remainder (n = 899) were obtained from fishery-independent sources.

Gray triggerfish samples ranged from 74 to 697 mm FL (mean 342.5 mm, SE $\pm$ 1.91; Figure 8), and integer ages ranged from 0 to 14 years (mean 4.1 years, SE $\pm$ 0.04; Figure 9). The smallest and youngest fish (75 mm FL, age-0) were captured in fishery-independent trawl surveys (Figure 8, C; and Figure 9, C), and the largest and oldest fish (697 mm FL, age-14) by the commercial long-line fishery (Figure 8, A; and Figure 9, A).

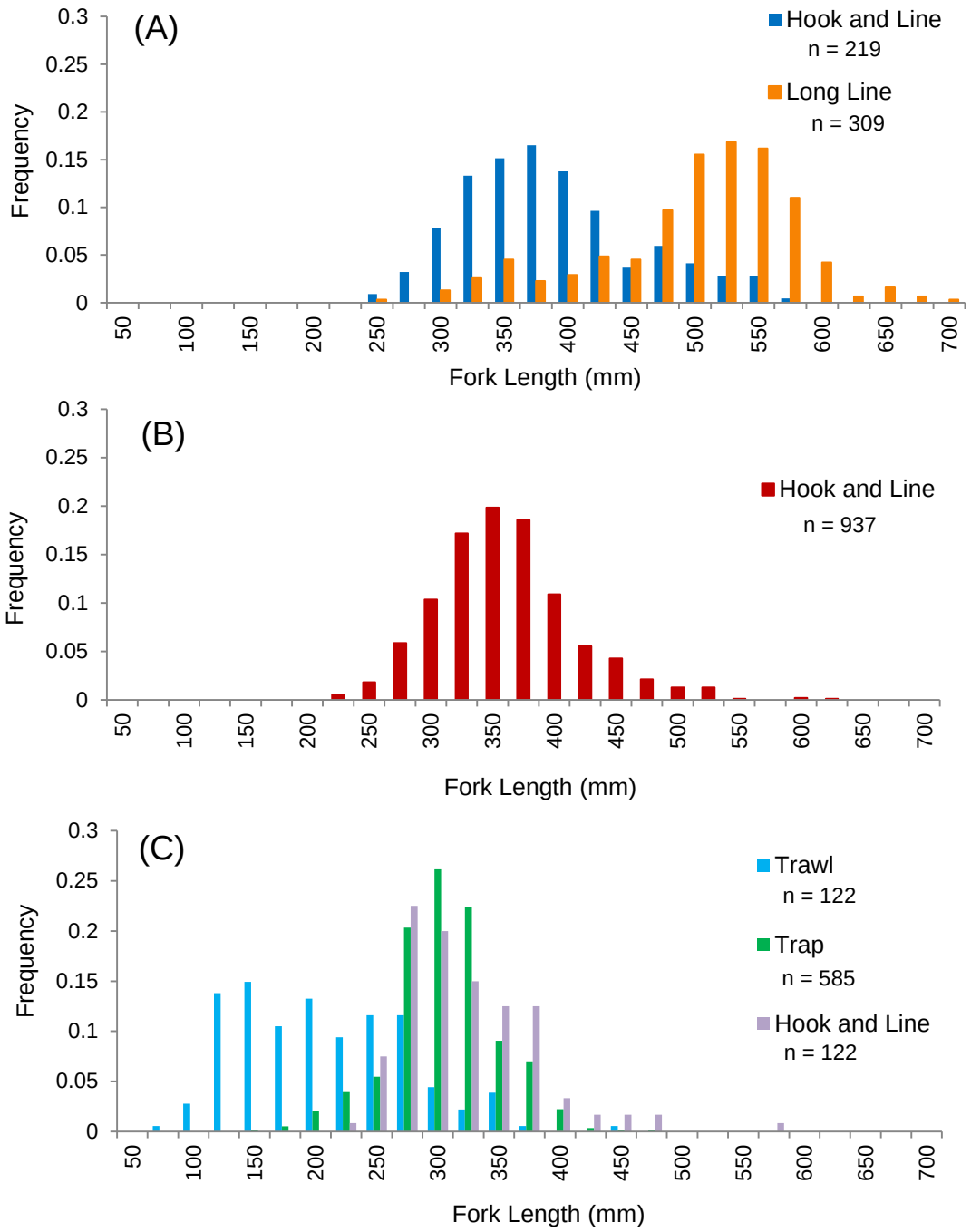


*Figure 8*.—Relative length frequency distribution for Gulf of Mexico (GOM) gray triggerfish for (A) commercial sources by hook and line and long line, (B) recreational sources by hook and line, and (C) fishery-independent sources by hook and line, trap and trawl gear types.

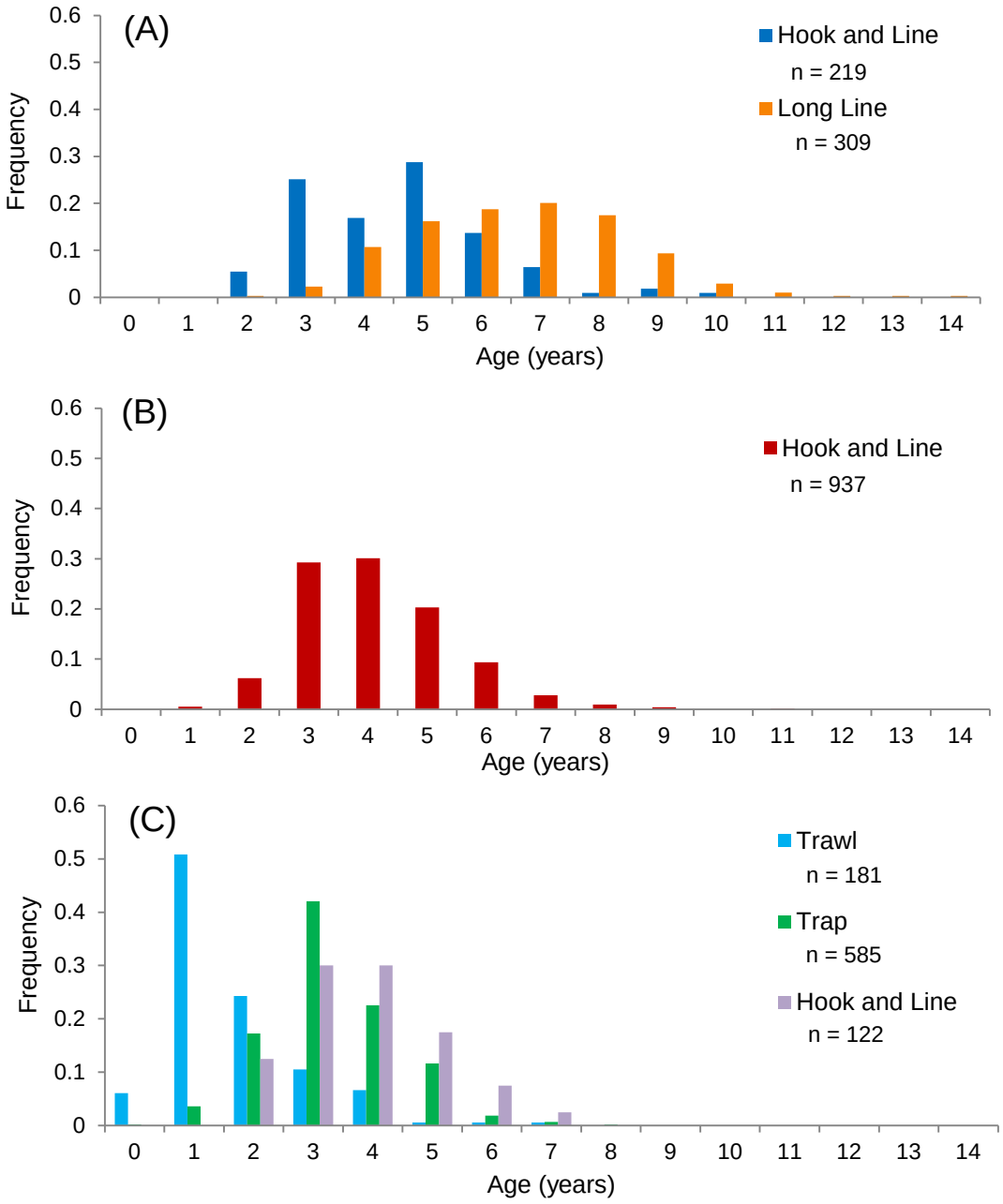


*Figure 9.*—Relative age distribution for Gulf of Mexico (GOM) gray triggerfish for (A) commercial sources by hook and line and long line, (B) recreational sources by hook and line, and (C) fishery-independent sources by hook and line, trap and trawl gear types.

Average Percent Error for primary and secondary readers with 100% overlap for GOM gray triggerfish dorsal spine ages (n = 2,348) was 10.8 %. The primary reader (author) tended to assign higher ages to younger fish and lower ages to older fish relative to the age assignments of the secondary reader (Figure 10). Marginal increment analysis for the two most common age classes indicates smaller mean monthly relative marginal increments existed from April to October in age-3 fish and from February through May in age-4 fish (Figure 11; A, B).

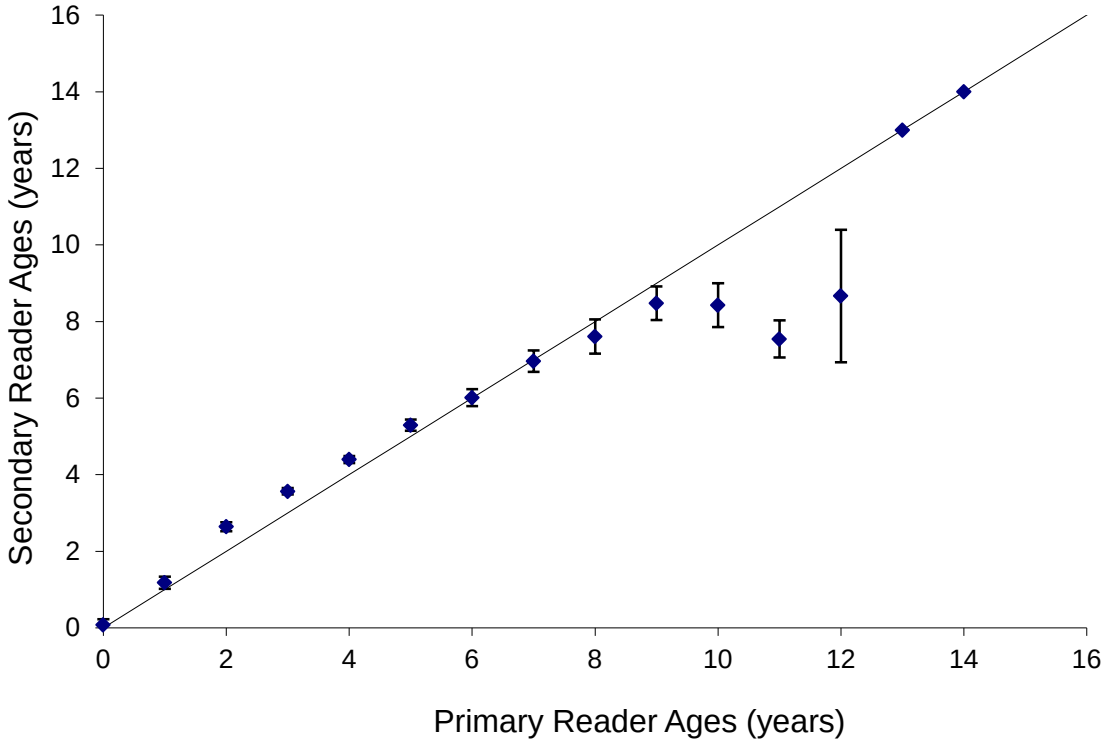


*Figure 10.*—Bias plot for primary reader (author) and secondary reader age estimates for Gulf of Mexico (GOM) gray triggerfish (n = 2,348). Age classes range from 0 to 14. Error bars represent 95% confidence intervals for secondary reader age estimates.

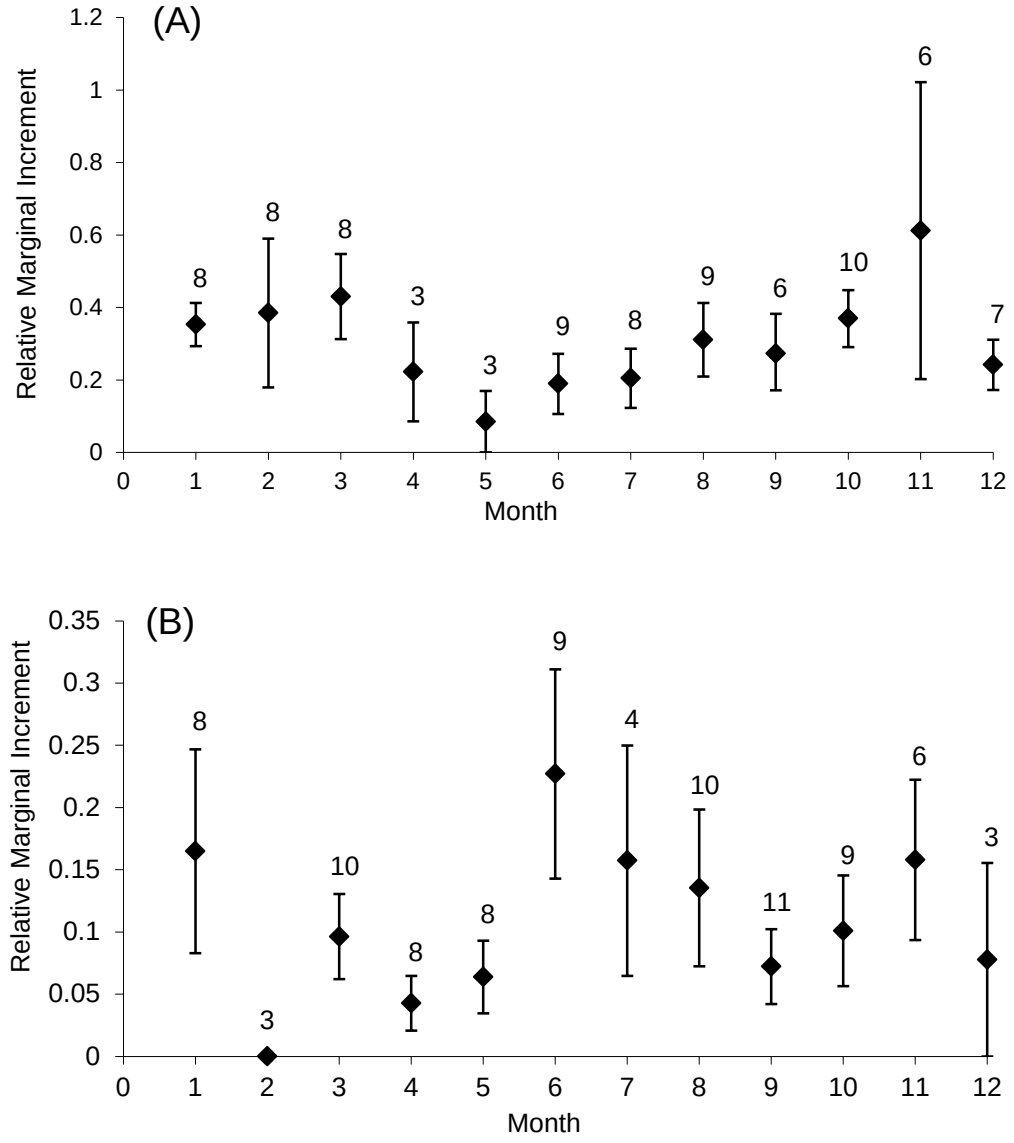


*Figure 11*.—Mean monthly relative marginal increment of (A) age-3 (n = 85) and (B) age-4 (n = 89) Gulf of Mexico (GOM) gray triggerfish sampled from the north central Gulf (NCG) study region. Error bars are standard error; monthly sample size provided.

*Hard-part Comparison*

Preparing and reading fin ray samples was time consuming and difficult. Translucent zones and opaque zones were apparent in fin rays as well as spines, and translucent and opaque zone counts were the same for many fish (Figure 12). The slope of the fitted regression of spine age versus fin ray age was not significantly different from one ($t_{df=1;25}$ = -0.162, P = 0.5637). However, a y-intercept of 1.01 means there was on average a one zone difference in counts between the two structures (Figure 13).

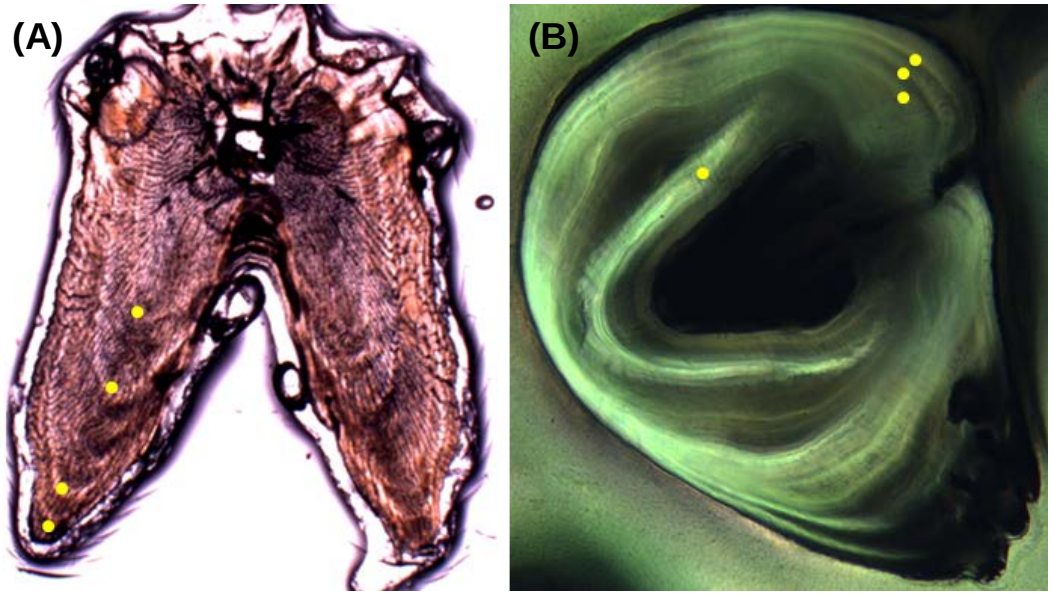


*Figure 12.*—Digital images of transverse (A) dorsal spine and (B) fin ray sections from a 249 mm fork length (FL) age-4 Gulf of Mexico (GOM) gray triggerfish. The spine magnification is 20x and it was viewed with transmitted white light, while the fin ray image was captured with 100x magnification and viewed with green filtered light. Yellow circles indicate translucent zones in each.

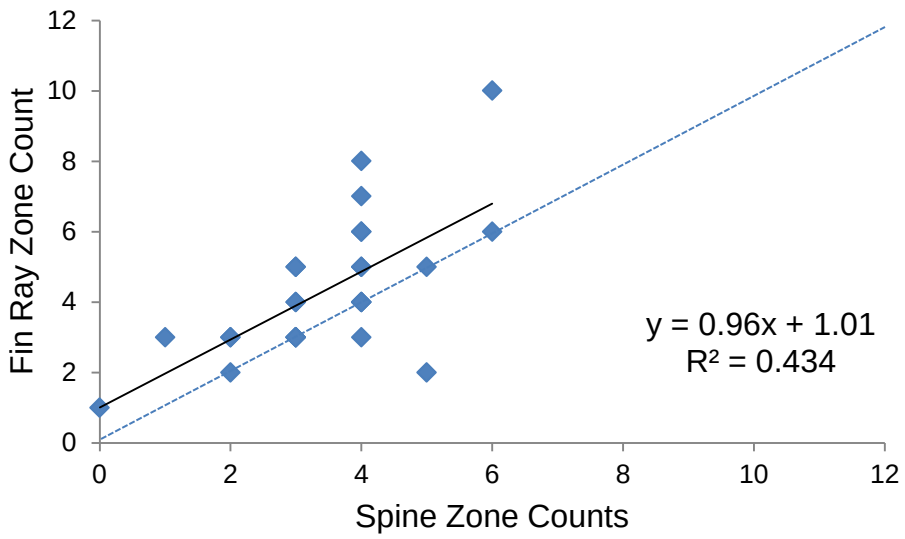


*Figure 13.*—Scatterplot of translucent zone counts in dorsal or pectoral fin rays versus dorsal spines of Gulf of Mexico (GOM) gray triggerfish. Solid line is a linear regression fit to the data ($R^2$ = 0.434; n = 27). Dotted line indicates the line of 1:1 agreement between counts.

*Growth Estimation and Variation in Size at Age*

There were clear differences in the VBGF's fit to only spine-based age data (Figure 14, A) versus spine-based and juvenile length frequency-based ages (Figure 14, B). The function that included the juvenile size at age estimates was anchored near the origin ($t_0$= -0.12 y) by the large number of young samples, while the function in which juvenile samples were excluded was not constrained to near the origin ($t_0$= -2.4 y). The VBGF that included juvenile data was more similar to previous VBGFs estimated for GOM gray triggerfish (Tables 1 and 2). No apparent difference existed between sex-specific VBGFs, but there were differences observed in fits between regions and among fisheries (Figure 15). Models for commercial and fishery-independent hook and line did not converge, therefore, are not depicted.

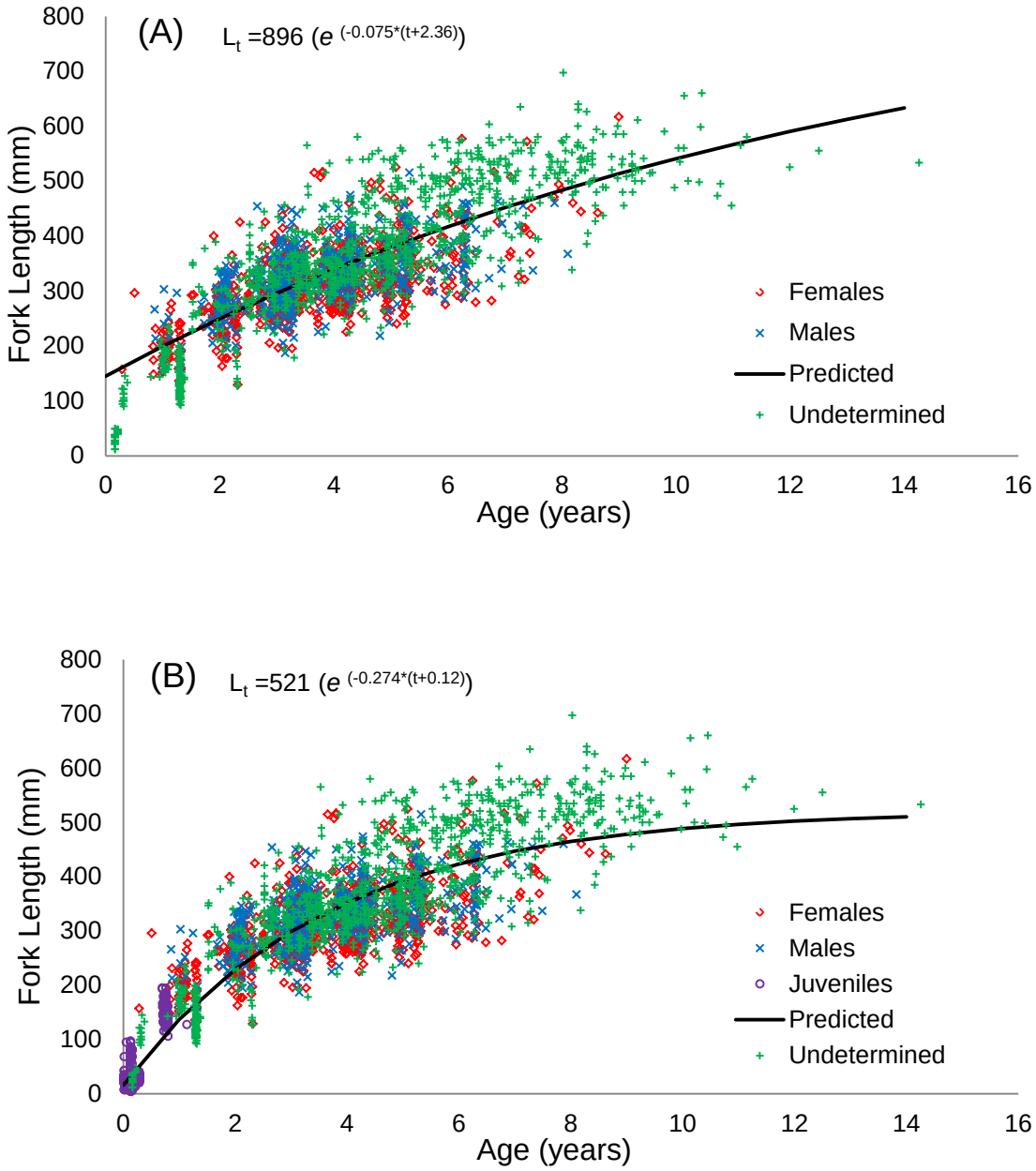


*Figure 14.*—Size at fractional age data for Gulf of Mexico (GOM) gray triggerfish captured from 2003 to 2010 and sampled from fishery-dependent and -independent sources and aged with first dorsal spines. The fitted line is the von Bertalanffy growth function (VBGF) fit to (A) all spine-based data combined (n = 2,391) (B) addition of length frequency data (n = 3,466).

*Table 1*.—Von Bertalanffy Growth Parameters for All Gulf of Mexico (GOM) Gray Triggerfish Combined and by Sex, Fishery, and Region. Fisheries: CM-LL = Commercial Long Line, REC-HL = Recreational Hook and Line, SS-TR = Fishery-independent Trap SS-TRW = Fishery-independent Trawl. Regions: NCG = North Central Gulf of Mexico, EG = Eastern Gulf of Mexico.

| VBGF | Spine-based Data | Including Juvenile Data | Female | Male | CM-LL | REC-HL | SS-TR | SS-TRW | NCG | EG |
|---|---|---|---|---|---|---|---|---|---|---|
| N | 2,391 | 3,466 | 1,967 | 1,640 | 309 | 934 | 585 | 123 | 1,422 | 854 |
| $L_\infty$ | 896 | 521 | 381 | 403 | 581 | 912 | 339 | 526 | 524 | 832 |
| k | 0.075 | 0.274 | 0.498 | 0.491 | 0.287 | 0.042 | 0.445 | 0.139 | 0.144 | 0.101 |
| to | -2.36 | -0.12 | -0.02 | < -0.01 | -0.39 | -7.36 | -1.40 | -2.07 | -3.11 | -1.53 |
| MSE | 3,516 | 2,883 | 1,571 | 1,208 | 3,441 | 2,297 | 1,436 | 1,600 | 2,570 | 3,764 |

*Table 2*.—Von Bertalanffy Growth Parameters for Gray Triggerfish from Previous Studies in the Northern Gulf of Mexico (N GOM; 1984-2001). Fork length = FL, Northeast Gulf of Mexico = NE GOM Northern Gulf of Mexico = N GOM.

| k | $L_\infty$ (mm) | $t_0$ (years) | Max Age (years) | Max Length (mm) | Sex | Region | Reference |
|---|---|---|---|---|---|---|---|
| 0.383 | 438 FL | 0.15 | 12 | 561 FL | female | NE GOM | Johnson and Saloman (1984) |
| 0.382 | 492 FL | 0.23 | 13 | 544 FL | male | NE GOM | Johnson and Saloman (1984) |
| 0.382 | 466 FL | 0.19 | | | pooled | NE GOM | Johnson and Saloman (1984) |
| 0.208 | 514 | -1.61 | 9 | 550-599 FL | female | N GOM | Ingram (2001) |
| 0.199 | 598 | -1.37 | 8 | 550-599 FL | male | N GOM | Ingram (2001) |
| 0.183 | 583 | -1.58 | | | pooled | N GOM | Ingram (2001) |
| 0.329 | 421 | -1.20 | 10 | 605 | female | NE GOM | Hood and Johnson (1995) |
| 0.156 | 645 | -1.80 | 14 | 725 | male | NE GOM | Hood and Johnson (1995) |
| 0.152 | 556 FL | -1.90 | | 725 | pooled | NE GOM | Hood and Johnson (1995) |

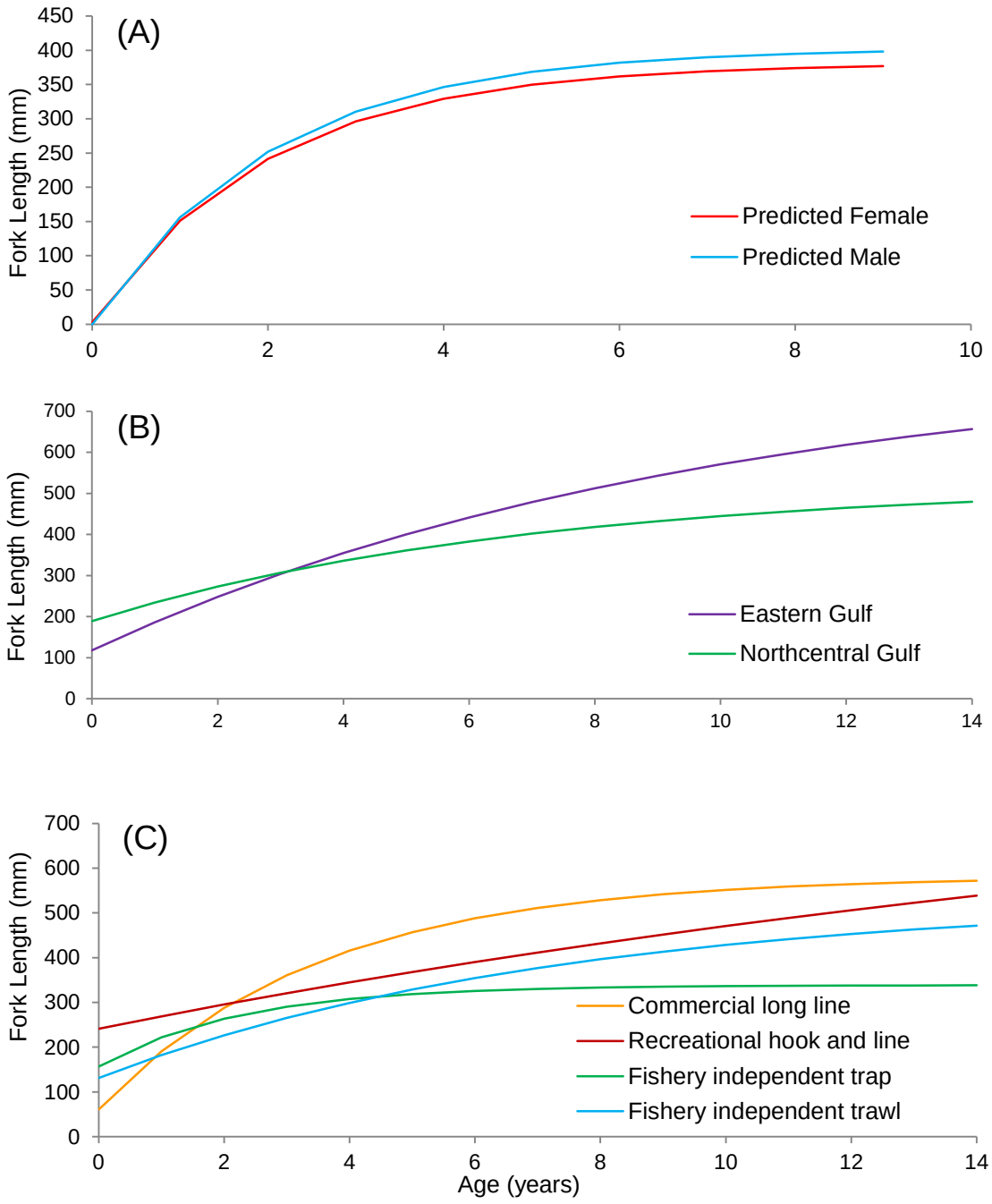


*Figure 15*.—Size at fractional age data for Gulf of Mexico (GOM) gray triggerfish captured from 2003 to 2010, sampled from fishery-dependent and -independent sources and aged with first dorsal spines. The fitted line is the von Bertalanffy growth function (VBGF) fit to (A) sexes: females (n = 894) and males (n = 567), (B) regions: north central Gulf (NCG; n = 1422) and eastern Gulf (EG; n = 854), and (C) fisheries: commercial long line (CM-LL; n = 309), recreational hook and line (REC-HL; n = 934), fishery-independent trap (SS-TR; n = 585), and fishery-independent trawl (SS-TRW; n = 123).

Results of a 3-way ANOVA for samples age-2 through age-8 (n = 2,173) indicate no significant difference in size at age between males and females (Table 3 and Figure 16). Sex ratios indicate that females outnumbered males greater than 1.6:1. Results of a chi-square goodness of fit test indicate that this ratio is significantly different from a 1:1 ratio (Table 4).

*Table 3*.—Three-way Analysis of Variance (ANOVA) Results for Gulf of Mexico (GOM) Gray Triggerfish by Sex, Fishery, and Region.

| Source | DF | Type III SS | Mean Square | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 2 | 12,311 | 6,156 | 2.09 | 0.124 |
| Region | 1 | 25,814 | 25,814 | 8.79 | 0.003 |
| Fishery | 5 | 470,587 | 94,117 | 32.00 | <.001 |
| Sex*Fishery | 7 | 4,165 | 595 | 0.99 | 0.985 |
| Sex*Region | 2 | 2,597 | 1,298 | 0.64 | 0.643 |
| Fishery*Region | 5 | 261,369 | 52,274 | 17.78 | <.001 |
| Sex*Fishery*Region | 6 | 28,891 | 4,149 | 1.41 | 0.207 |
| Error | 2,144 | 6,306,430 | 2,941 | | |
| Corrected Total | 2,172 | 14,862,639 | | | |

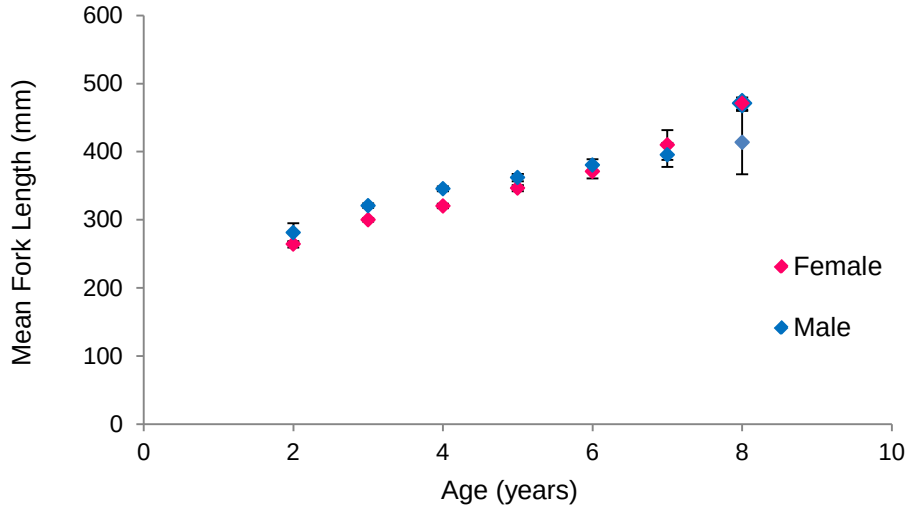


*Figure 16*.—Mean fork length (FL) at age for female (n = 886) and male (n = 565) Gulf of Mexico (GOM) gray triggerfish aged with dorsal spines. Error bars are standard error.

*Table 4*.—Sex Ratio Data for Gulf of Mexico (GOM) Gray Triggerfish Landed by Recreational Hook and Line Fishery and Fishery-independent Surveys. Chi-square ($\chi^2$) Goodness of Fit Results Significantly Different from 1:1 for All Groups ($p < 0.05$). North Central Gulf = NCG, Eastern Gulf = EG.

| | NCG | EG | Both |
|---|---|---|---|
| Females (n) | 655 | 236 | 891 |
| Males (n) | 430 | 130 | 560 |
| sex ratio | 1.5:1 | 1.8:1 | 1.6:1 |
| $\chi^2$ | 46.7 | 30.7 | 75.5 |
| p-value | <0.001 | <0.001 | < 0.001 |

There were significant differences in size at age among fisheries and between regions (Table 3 and Figure 17; A, B), but a significant interaction between them confounds interpretation of main effects (Figure 18). This interaction may be an artifact of sample sizes or differences in fishing practices between fisheries and regions (Tables 5 and 6).

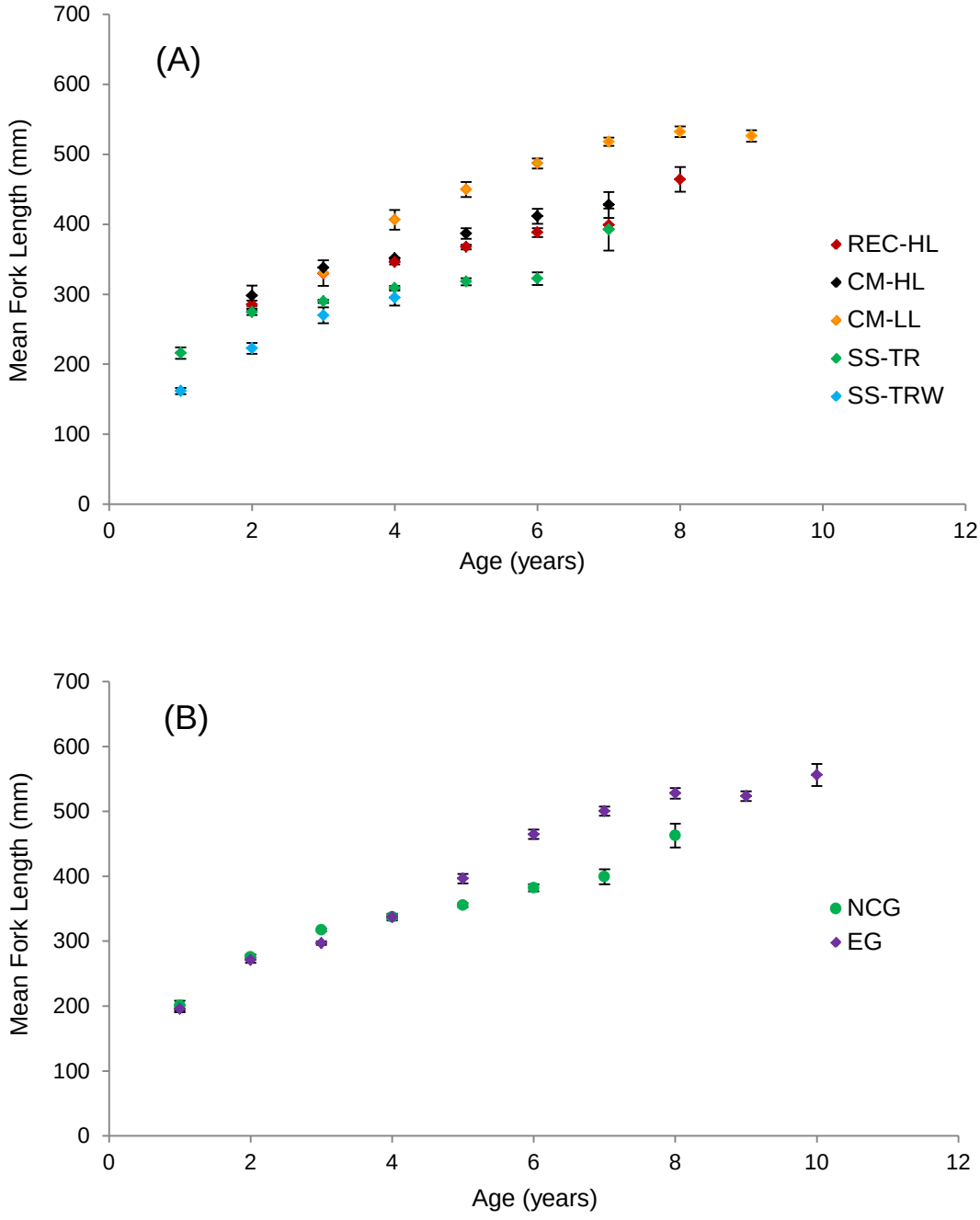


*Figure 17*.—Gulf of Mexico (GOM) gray triggerfish mean size at age (n > 5) for (A) fishery: recreational hook and line (REC-HL), commercial hook and line (CM-HL), commercial long line (CM-LL), fishery-independent trap (SS-TR), fishery-independent trawl (SS-TRW) and (B) region: north central (NCG) and eastern (EG) Gulf study regions. Error bars are standard error.

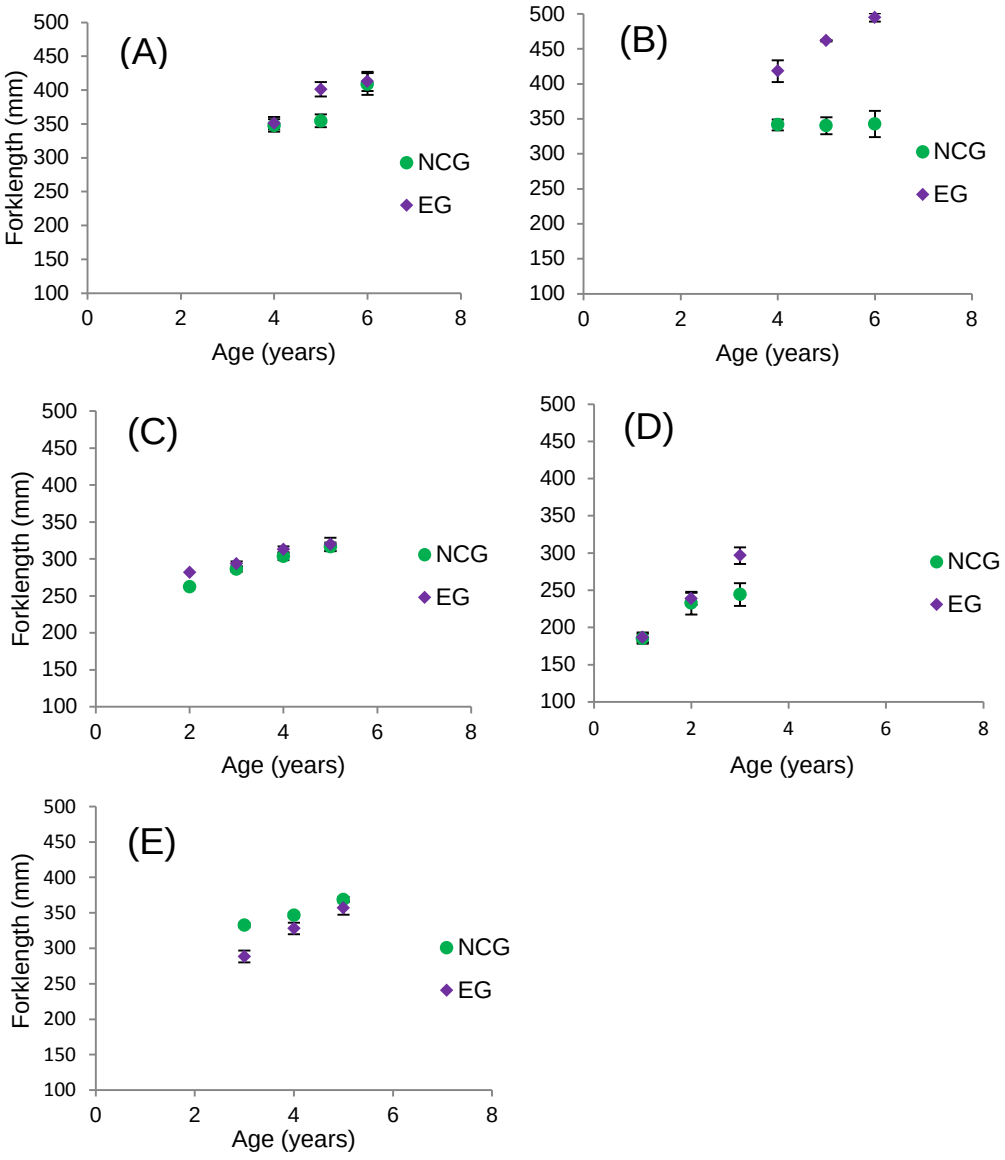


*Figure 18*.—Gulf of Mexico (GOM) gray triggerfish mean size at age for each fishery by north central (NCG) and eastern (EG) Gulf study regions: (A) = commercial hook and line (CM-HL), (B) = commercial long line (CM-LL), (C) = fishery-independent trap (SS-TR), (D) = fishery-independent trawl (SS-TRW), (E) = recreational hook and line (REC-HL). Error bars are standard error.

*Table 5*.—Sample Size for Gulf of Mexico (GOM) Gray Triggerfish by Fishery (Commercial: Long Line = CM-LL and Hook and Line = CM-HL, Recreational: Hook and Line = REC-HL and Spear = REC-SP, Fishery-independent: Hook and Line = SS-HL, Trap = SS-TR, Trawl = SS-TRW) and Age from North Central Gulf (NCG) Study Region.

| Fishery | Age:0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CM-HL | | | 11 | 46 | 21 | 20 | 10 | 3 | 1 | | | | | | | 112 |
| CM-LL | | | 1 | 3 | 5 | 5 | 3 | | 1 | | | | | | | 18 |
| REC-HL | | 5 | 50 | 255 | 263 | 172 | 82 | 23 | 8 | 4 | | 1 | | | | 863 |
| REC-SP | | | | 2 | 2 | | | | | | | | | | | 4 |
| SS-HL | | | 15 | 33 | 35 | 19 | 9 | 3 | | | | | | | | 116 |
| SS-SP | | | 2 | 7 | 1 | 1 | | | | | | | | | | 11 |
| SS-TR | | 4 | 39 | 114 | 60 | 42 | 10 | 3 | | | | | | | | 272 |
| SS-TRW | | 25 | 12 | 6 | | | | | | | | | | | | 43 |
| Total | | 34 | 131 | 467 | 387 | 259 | 114 | 32 | 10 | 4 | | 1 | | | | 1,439 |

*Table 6*.—Sample Size for Gulf of Mexico (GOM) Gray Triggerfish by Fishery (Commercial: Long Line = CM-LL and Hook and Line = CM-HL, Recreational: Hook and Line = REC-HL and Spear = REC-SP, Fishery-independent: Hook and Line = SS-HL, Trap = SS-TR, Trawl = SS-TRW and Age from Eastern Gulf (EG) Study Region.

| Fishery | Age:0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CM-HL | | | 1 | 8 | 13 | 37 | 17 | 10 | 1 | 4 | 2 | | | | | 96 |
| CM-LL | | | | 4 | 28 | 45 | 55 | 62 | 53 | 29 | 9 | 3 | 1 | 1 | 1 | 291 |
| REC-HL | | | 8 | 19 | 19 | 16 | 5 | 3 | 1 | | | | | | | 71 |
| REC-SP | | | | 1 | 8 | 4 | 5 | | | | 1 | 1 | | | | 20 |
| SS-HL | | | | 3 | 1 | 2 | | | | | | | | | | 6 |
| SS-TR | 1 | 17 | 62 | 132 | 72 | 26 | 1 | 1 | 1 | | | | | | | 313 |
| SS-TRW | 2 | 31 | 22 | 11 | 11 | 1 | 1 | 1 | | | | | | | | 80 |
| Total | 3 | 48 | 93 | 178 | 152 | 131 | 87 | 77 | 56 | 33 | 12 | 4 | 1 | 1 | 1 | 877 |

# DISCUSSION

The most significant result of this study was the direct validation of annual translucent zone formation in gray triggerfish dorsal spines. Experimental fish were injected with OTC in fall and a translucent zone formed in spines during the winter following OTC injection. Perhaps it would have been better to inject experimental fish with OTC earlier in the season (e.g., August or September) during the period of highest growth, hence middle of an opaque zone. Holding experimental fish for a second year likely would have provided even more robust results, as would marking tagged fish to be released into the wild for subsequent recapture. However, results from the OTC marking experiment, as it was conducted, clearly validate one translucent zone being formed in winter.

Similar to spines, fin ray translucent zones were validated as being formed in winter with OTC marking. However, there was considerably more variance in fin ray translucent zone counts than in spines. Therefore, spines should be viewed as the preferred hard-part with which to age gray triggerfish. Typically, fin rays were assigned a higher age (an average of 1.01 higher as illustrated by the y-intercept) than spines. This may be due to my inexperience in aging gray triggerfish fin ray sections, specifically the problem of first annulus identification (i.e. first counted growth zone looked distinctly different from others and may have been either part of the core or a settlement mark). Examination of fin ray sections from individuals of age-0 and age-1 would facilitate

identification of the first annulus. Preparation and processing of the fin rays also was laborious due to their small size. Furthermore, sample extraction to produce a readable transverse section of a fin ray required removal of the structure at the insertion into the pterygiophore increasing the potential for infection, and possibly death of the fish. Invasiveness of the extraction may preclude use of fin rays for a non-lethal means of age determination.

While results of the OTC marking experiment provide clear validation of a single translucent zone being formed in spines during winter, the verification of annual translucent zone formation in wild fish provides equally meaningful results with respect to the efficacy of aging gray triggerfish with dorsal spines. Results from relative marginal increment analysis conducted for age-3 and age-4 fish further verify annual translucent zone formation in gray triggerfish dorsal spines. Relative marginal increments were smaller during the spring and summer months due to translucent zone deposition during the winter and early spring months. Ingram (2001) reported a similar result for fish captured off Alabama in the late 1990s, but relative marginal increment plots in the current study provide even more compelling evidence of annual translucent zone formation.

Aging precision (i.e., reproducibility of ages) is another important aspect of age determination which has implications for examining a fish's ecology as well as conducting age-based stock assessments for fishery species (Campana 2001). If ages are produced by different readers or laboratories, then quality control protocols are required to measure precision among readers or groups. In the current study, the metric APE was computed to assess between reader aging precision for spine-based gray triggerfish ages

(Beamish and Fournier 1981). The computed APE of 10.8% would be considered a moderate APE based on the definitions of Campana (2001). While programs providing ages for age-based stock assessments typically strive for APE <5% for moderately difficult to age species, most aging of bony fishes is done with otoliths which are typically much easier to analyze than triggerfish spines which would be considered relatively difficult to age.

The observed age structure for GOM gray triggerfish was similar to that reported by Johnson and Saloman (1984) and Hood and Johnson (1997), with the oldest fish estimated at 14 years and the predominant age classes ranging from 3-5 years. The oldest observed male in this study was 8 years old and the oldest female at 9 years old, similar to those reported by Ingram (2001) but lower than those reported by Hood and Johnson (1997) and Johnson and Saloman (1984). However, 18 fish >10 years old were observed, but no sex data was available given they came from the commercial long line fishery where fish are gutted at sea; thus, gonads are not present when fish are landed.

There have been previous attempts to age gray triggerfish in the GOM, but this study is the most comprehensive to date (Johnson and Saloman 1984; Hood and Johnson 1995; Ingram 2001). Despite the large sample size analyzed in this study, relatively few fish <2 years old were available. The largest gray triggerfish in this study and other studies (Hood and Johnson 1997) and testimonials by divers and anglers do not support fish exceeding a FL of approximately 700 mm. Thus, VBGFs fit only to spine-based age data had parameters that were not biologically realistic. The resultant function is due to a

lack of samples at the extremes of the age distribution (i.e., few small or large fish) and does not effectively describe gray triggerfish growth in GOM.

A much more realistic fit was obtained when juvenile samples whose age was estimated from length were included in the VBGF. Age assignment was fairly straight forward for these juveniles, as fish collected in the *Sargassum* are young of the year that have not yet settled to reefs (Wells and Rooker 2004). Fish collected in bottom trawls have settled onto reefs during the first/winter of life; therefore, settled juveniles could be age-0 or age-1. However, the bimodal distribution of the data made it easy to distinguish age-0 from age-1 fish. Overall, the addition of the large number of small, young fish included in the model improved the fit to the data as $t_0$ was constrained at the origin. However, $L_\infty$ was slightly below the anticipated maximum asymptotic length with a greater proportion of positive than negative residuals due to the least squares fit to minimize error. The addition of larger, older fish to the growth curve would have further improved the fit and more effectively described growth of gray triggerfish in the GOM.

Variation in estimated growth parameters has been historically high for gray triggerfish (Johnson and Saloman 1984: Hood and Johnson 1997: Ingram 2001). Sampling differences (e.g., location and gear) between this and previous studies may have driven some of the differences in estimated VBGF parameters. Johnson and Saloman (1984) obtained samples from the hook and line fishery off of the coast of Panama City, Florida. Ingram (2001) sampled fish from the hook and line fishery and tournaments, as well as fishery-independent trawl samples, off the coast of Dauphin Island, Alabama. Hood and Johnson (1997) obtained samples from head boats, commercial fisheries, and fishery-independent trawl surveys from the EG. Utilization of

too few gear types causes truncation of the data due to gear selectivity. Gear selectivity also can lead to biased growth estimates, as highly selective fisheries will remove the fastest growing individuals first as they recruit to the gear (Goodyear 1995).

Growth parameter estimates from other studies may be biased due to their limited sampling areas or gear types. While Hood and Johnson (1997) sampled from both fishery-dependent and fishery-independent sources and a modest geographic region, the growth parameters estimated in this study differed in terms of Brody's growth coefficient (k) and age at length zero ($t_0$) estimated from the model incorporating juvenile data. Johnson and Saloman (1984) had a lower $L_\infty$ estimate, likely due to the fact that sampling occurred within a small geographical region off of the coast of northwest Florida, as well as gear type being limited to hook and line, which catches smaller fish than long lines. On the other hand, Ingram (2001) reported a higher $L_\infty$ estimate than the current study, which could likely be for the same reason as limited geographical location off the coast of Alabama and inclusion of tournament fish in his model. My work incorporates samples from a wider geographic region and all possible gear types; therefore, growth parameters estimated can be considered more comprehensive and may better represent growth of NCG and EG fish than previous studies.

It has been reported that gray triggerfish exhibit different growth for males and females (Johnson and Saloman 1984; Wilson et al. 1995; Harper and McClellan 1997; Hood and Johnson 1997; Ingram 2001; Moore 2001), with males attaining a larger asymptotic length than females. However, Ingram (2001) was the only author to compare growth function parameters between sexes and find a statistically significant difference. Slight differences in VBGF parameters were observed between the sexes in the current

study, with males having a higher $L_{\infty}$ and lower k than females, but mean size at age was not significantly different between sexes. The juvenile data comprised a large proportion of data in the model and may have been responsible for the similarities in the growth functions.

Ingram (2001) inferred that neither males nor females feed during spawning events. However, direct observation of nesting gray triggerfish only provides evidence that females do not feed (Makichan and Szedlmayer 2007). Cessation of foraging by the female is likely required for a short duration for nest defense and selected for within the species as it improves reproductive fitness. If this behavior is exhibited by the female alone, it may add to growth differences between the sexes, as she would refrain from foraging for up to two days immediately after producing a clutch of eggs (i.e. high energy expenditure). Whether or not males refrain from foraging is more difficult to discern. Males are required to make a high investment in terms of spending time and energy patrolling their territory, building additional nests, and attracting females. One piece of evidence to support the hypothesis of cessation of male foraging during nesting can be inferred by considering a unique anatomical feature of male gray triggerfish, an accessory gland adjacent to the testes (Moore 2001: SEDAR 2012). This may indicate that the male is conserving some energy by storing sperm rather than producing it in order to fast. However, this adaptation may simply allow the male to mate with many females simultaneously by allowing him to store sperm as it increases his ability to fertilize the eggs. Conversely, males may continue to forage as the high energetic costs required to support the observed reproductive behaviors maximize his fitness (Makichan and Szedlmayer 2007).

The fact that females outnumbered males 1.6:1 may be a result of the polygynous reproductive strategy employed by the species and/or the accompanying aggressiveness of the male during spawning season leading to higher total mortality for males. Males and females also may have different natural mortality as indicated by sex ratios and longevity, (Johnson and Saloman 1984; Hood and Johnson 1997). Having more females with smaller clutches of protected eggs (with multiple spawns per year) may be a successful reproductive strategy. Also, fishing mortality may not be an issue in terms of reproductive success, as both sexes appear to be resistant to capture during nesting (Ingram 2001).

One objective of this study was to examine regional differences in size at age or growth to infer population structure differences among regions. Antoni et al. (2011) reported there were no genetic differences in GOM gray triggerfish among my study regions, but lack of genetic differences does not preclude differences in population demographics (i.e. phenotypic expression of genotypic attributes in concert with environmental factors) from being present (Begg and Weldman 1999). In fact, Ingram (2001) reported spatial scale differences in growth in the northern GOM as the VBGF for fish sampled off Alabama had a higher $L_{\infty}$ and lower k than for fish sampled off the coast of Panama City, Florida (Johnson and Saloman 1984). However, these growth parameter differences also may be attributed to the inclusion of tournament caught fish in the Alabama region or temporal differences in growth (Ingram 2001).

Detection of differences in size at age among regions was confounded in the current study due to differences in the predominant fisheries from which fish were sampled. Gear selectivity may have been the primary reason for differences in size at age

among regions, as well as the significant interaction between region and fishery effects in the ANOVA model. For example, more fish were sampled from commercial long line landings in the EG than in the NCG, and large hooks used in that fishery may have selected for large fish and likely fast growers. It is also plausible that fish captured by commercial long line fishers in the EG were larger in size at age due to differential fishing pressure between regions. Populations can undergo a decline in size at age due to fishing pressure, as faster growing fish recruit to the gear before their slower growing counterparts, allowing the slow growers to have more reproductive success, thus selecting for slower growth in that population (Ricker 1981; Harris and McGovern 1997; Zhao et al. 1997). However, it has also reported that exploitation of fish stocks can have the opposite effect (i.e. inducing faster growth of individuals) due to density-dependence, with removal of part of the population by fishers decreasing competition for resources and thereby increasing growth (Sinclair et al. 2002).

Variation in growth on small spatial scales may further confound detecting differences on a larger, regional scale. Ingram (2001) reported differences in gray triggerfish growth on small spatial scales (10s of km) which he attributed to the patchy distribution of reef habitat and high site fidelity of gray triggerfish (Ingram 2001; Addis et al. 2008), and density-dependent growth effects detected among his sample reefs (Lindberg et al. 2006).

Estimates from past and current stock assessments for GOM gray triggerfish have indicated the stock is overfished and undergoing overfishing (SEDAR 2006, 2012). More research on the population ecology of this species is needed to better define and manage the stock. Alternatives to typical stock management strategies, such as season closures

and marine protected areas (versus bag limits and minimum size limits) may be a viable beneficial for this species. High site fidelity and wide distribution of larvae and juveniles during the extended early, life history of gray triggerfish may make it a good candidate for inclusion in Marine Protected Areas (MPA) (Ingram and Patterson 2001). However, strategic placement of an MPA in source rather than sink areas will likely contribute greatly to the benefits of MPA creation (Crowder et al. 2000).

Currently, gray triggerfish are managed as a single stock in the GOM (SEDAR 2006). Management units or stocks have been described as having similar life history parameters and fishing exploitation (Begg and Waldman 1999). Having knowledge of exchange rates between subpopulations and the existence of metapopulations is another important component of fisheries management (e.g., avoidance of depletion of local populations and implementation of inappropriate targets and erroneous levels of harvest is dependent on those dynamics) as ignorance of spatial structure may be detrimental to stocks (Ying et al. 2011). Even though differences in growth parameters may exist on small spatial scales (Ingram 2001), unless these differences exist among larger geographical regions, managing GOM gray triggerfish as a single stock is the most effective and practical management strategy. The question of whether northern GOM gray triggerfish constitute a marine metapopulation will remain a subject for future research.

My work provides evidence that differences in population demographics on a regional scale may exist and these differences must be investigated through the use of effective experimental design in order to make a meaningful comparison between regions. A fishery-independent study that incorporates sampling on a broad spatial scale,

and most importantly, with all gear types (e.g. neuston nets, trawls, hook and line, and long line) may produce a more powerful result without confounding interactions among main effects. Another benefit to sampling fish through fishery-independent means is that sex data for the largest fish would not be lost as it was here due to the fact that commercial fish are gutted at sea.

In the end, the most significant contribution of this study is the validation and verification of annual translucent zone formation in gray triggerfish spines. Others have used spines to age triggerfish in previous studies (Johnson and Saloman 1984; Wilson et al. 1995; Harper and McClellan 1997; Hood and Johnson 1997; Ingram 2001; Moore 2001), but until now annual formation of translucent zones was only assumed. Validation of this aging approach is critical for studying gray triggerfish population ecology, as well as for age-based stock assessment of this species.

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# APPENDIX

# Appendix A

## Institutional Animal Care and Use Committee Approval

***MEMORANDUM***

April 11, 2012

To: Dr. Richard Podemski, Graduate Dean

Carrie Fioramonti, graduate stduent

From: Dr. George Stewart, Chair
Institutional Animal Care and Use Committee

Subject: Approval of protocol employed in thesis project

Carrie Fioramonti, a graduate student in Biology studying under Dr. Will Patterson, has completed her thesis project which was conducted under the auspices of the NOAA Fisheries Laboratory in Panama City, FL. Several years ago the UWF IACUC was struggling with how to conduct oversight and approval of projects involving fish. There were no clear guidelines available and we noted that state and federal agencies involved in this type of work had their own sets of guidelines under which researchers funded by said agencies were bound. Several of our researchers were funded by these agencies. Therefore, their research and that of their grad students came under the guidelines dictated by these agencies and we decided to defer to those agency guidelines and requested copies of such guidelines be filed with IACUC as evidence that agency-funded research conducted by our faculty and their grad students was in compliance with a set of state/federal standards. Carrie's thesis research on fish falls within this category and was funded by and conducted under NOAA guidelines for the ethical and humane treatment of research animals established by this agency. The IACUC considers this thesis project approved and views the research in compliance with federal standards for the care and use of fish.