AGE, GROWTH, AND REPRODUCTION OF GRAY TRIGGERFISH *Balistes capriscus* OFF THE SOUTHEASTERN U.S. ATLANTIC COAST

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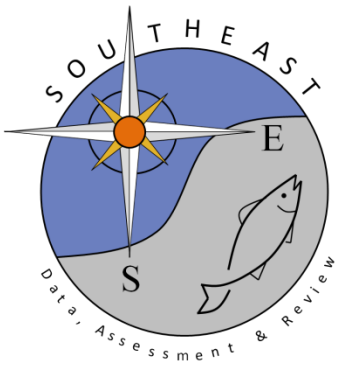

AGE, GROWTH, AND REPRODUCTION OF GRAY TRIGGERFISH *Balistes capriscus* OFF THE SOUTHEASTERN U.S. ATLANTIC COAST

A thesis submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

in

MARINE BIOLOGY

by

AMANDA M. KELLY
APRIL 2014

at

THE GRADUATE SCHOOL OF THE COLLEGE OF CHARLESTON

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ABSTRACT

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Gray Triggerfish *Balistes capriscus* is an economically valued reef fish species, managed by the South Atlantic Fishery Management Council as part of the snapper-grouper complex fishery. Over the past twenty years, commercial landings cumulatively approached over 7,000,000 lbs in U.S. Atlantic waters. Despite the economic importance of this species, no peer-reviewed published information exists concerning age, growth, and reproduction in U.S. Atlantic waters. Fishery-independent samples were utilized to assess life history parameters of the Gray Triggerfish population off the southeastern U.S. Atlantic coast during 1991-2012. Specifically, temporal variation in life history parameters was determined by comparing parameters between 1994-1997 and 2009-2012. Specimens were assigned ages from increment counts on the first dorsal spine. Sex and reproductive state were determined from histological sections of the gonad. Males were significantly larger than females (337 and 304 mm FL, respectively). Mean ages were significantly different between males and females (3.7 years and 3.4 years, respectively). Female spawning season occurred from April to September with a peak spawning period from May to August. Mean lengths of males and females significantly increased between the two time periods. These results provide essential life history information for any future stock assessments and have the potential to affect future management decisions of the Gray Triggerfish population off the southeastern U.S. Atlantic coast.

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INTRODUCTION

Gray Triggerfish *Balistes capriscus* is a commercially and recreationally valued reef fish species. From 1990 to 2012, commercial landings have cumulatively reached over 7,000,000 lbs. off the southeastern U.S Atlantic coast (Figure 1). Gray Triggerfish are managed by the South Atlantic Fishery Management Council (SAFMC) as part of the South Atlantic snapper-grouper complex fishery. Currently, Gray Triggerfish are included in the 20 fish snapper-grouper aggregate daily bag limit off the southeastern U.S. Atlantic coast. In 1995, a 12 inch total length (TL) minimum size limit was put into effect off the Atlantic coast of Florida. Since the late 1990s, fishery-independent catch per unit effort (CPUE) for this species has fluctuated between an average of 0.12 to 1.20 fish per chevron trap per hour soak time (Figure 1). Many other species in the snapper-grouper complex, including Red Snapper and Gag Grouper, are considered overfished and are tightly regulated which has led to increased fishing pressure on alternative species including Gray Triggerfish. As of the writing of this thesis, stock status of Gray Triggerfish off the southeastern U.S. Atlantic coast has not been determined.

Despite the economic importance of Gray Triggerfish in U.S. Atlantic waters, no peer-reviewed published information exists concerning age, growth, and reproductive biology in this region. However, a thesis by Moore (2001) documented the life history of Gray Triggerfish off the southeastern U.S. Atlantic coast from 1992-1997 based primarily on fishery-independent samples provided by the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) program at the South Carolina Department of Natural Resources.

The Southeast Reef Fish Survey (SERFS), which is composed of MARMAP, the Southeast Area Monitoring and Assessment Program of the South Atlantic (SEAMAP-SA), and the Southeast Fishery-Independent Survey (SEFIS) program have been collecting life history data for this species off the southeastern U.S. Atlantic coast, an area of continental shelf ranging from Cape Hatteras, NC, to Port St. Lucie, FL, as part of its reef-fish monitoring program. MARMAP began the current chevron trap survey in 1988 and SEAMAP-SA and SEFIS joined the survey in 2009. Such long-term efforts and the resulting information are essential to providing a comprehensive assessment of the stock status of Gray Triggerfish in this region.

Gray Triggerfish have been reported along both sides of the Atlantic, as far north as Nova Scotia (Briggs 1958) and the North Sea (Harmelin-Vivien and Quero 1990) and as far south as Argentina (Briggs 1958) and Angola (Harmelin-Vivien and Quero 1990). Adults are typically associated with natural and artificial reefs, rocky outcroppings/hard bottom, and wrecks. Adults diurnally feed on invertebrate prey such as molluscs, crustaceans, and echinoderms (Frazer *et al.* 1991, Vose and Nelson 1994, Blitch 2000). Gray Triggerfish exhibit a relatively unusual mating strategy compared to other reef fish species in the snapper-grouper complex. Harem-like reproductive behavior has been observed in which males construct demersal nests and perform courtship behaviors (e.g., change in color and circling females) to attract multiple females with which to mate (Simmons and Szedlemayer 2012). After fertilization, parental care of the demersal eggs has been observed for both sexes. Typically, a female stays inside the nest and guards the eggs while the male guards the territory surrounding the nests. These behaviors continue until the eggs hatch, which occurs within 24-48 hours after fertilization (Simmons and

Szedlemayer 2012). During the early life stages, pelagic larvae and juvenile Triggerfish are associated with *Sargassum* spp. (Dooley 1972, Wells and Rooker 2004, Casazza and Ross 2008).

Prior to this study, the main sources of Gray Triggerfish life history information in the U.S. came from one peer-reviewed study (Johnson and Saloman 1984), a NOAA project report, and several Master's theses. Johnson and Saloman (1984) described age, growth, and mortality of Gray Triggerfish collected in 1979-1982 from the hook-and-line fishery off Panama City, Florida. Escorriola (1991) reported on age and growth from fish caught in the North Carolina recreational and commercial fisheries in 1989. Hood and Johnson (1997) documented age, growth, and aspects of reproduction in fish from the eastern Gulf of Mexico caught in the recreational and commercial fisheries from 1995-1996. Moore (2001), the previously mentioned thesis, quantified age, growth and reproduction of fish collected from 1992-1997 for fishery-dependent and -independent samples primarily off the southeastern U.S. Atlantic coast. Ingram (2001) examined age, growth, and reproduction for Gray Triggerfish from offshore Alabama fishery-dependent samples. The most recent study (Fioramonti 2012) described age and growth of fish from fishery-dependent and -independent sources throughout the northern Gulf of Mexico.

The purpose of this study was to utilize fishery-independent samples in order to 1) determine the population age and size structure and growth and 2) estimate spawning season and frequency, adult sex ratios, and size- and age-at-maturity of the Gray Triggerfish population off the southeastern U.S. Atlantic coast during 1991-2012. To determine if any shifts have occurred in the size structure, size-based sex ratios, and length at 50% maturity in response to increases in fishing pressure of the Gray

Triggerfish population off the southeastern U.S. Atlantic coast, these parameters were examined for two time periods - 1994-1997 and 2009-2012 (Appendix Table 1).

METHODS

Sampling.—Gray Triggerfish were collected by SERFS in chevron traps from 1991 to 2012, and processed for age and reproduction (n = 7685). Sampling efforts were conducted solely by MARMAP through 2009 and by the three partner programs, MARMAP, SEAMAP-SA, and SEFIS, from 2009 to the present day. Chevron traps were deployed during daylight hours in depths ranging from 10-110 m at reef sites randomly chosen from a universe of known live-bottom habitat sites. From 1991 to 2012, there were approximately 2500 live bottom sites, from which 300-900 randomly chosen sites were sampled each year since 1990 (Figure 2). Traps were baited using cut fish (mainly clupeids). Depth, latitude, longitude, sampling duration, and time of collection were recorded for each trap set. Gray Triggerfish was a relatively common fish species caught in the chevron traps and was sub-sampled for life history. For life history data, each Gray Triggerfish in the sub-sampled set was weighed to the nearest gram and measured for standard length (SL), fork length (FL), and total length (TL) to the nearest mm.

Age and growth.—The first dorsal spine is currently the accepted structure for estimating age in Gray Triggerfish (VanderKooy 2009, Fioramonti 2012). The spine was removed from the fish, cleaned of excess tissue, and stored dry until further processing. Two sections, immediately distal to the condyle groove (Figure 3), were cut from each spine (~0.7 mm thickness) using a low-speed saw with a diamond-edged double blade system

then mounted on glass slides with Cytoseal™ and viewed using a dissecting microscope at 10-20x magnification using transmitted light. For this study, age was assigned for fish samples from 2009-2012. Increment count was determined by identifying and enumerating the pattern of faster- and slower-growing zones (opaque zones and translucent zones, respectively) assumed to represent peak growth and non-growth seasons equating to one year of life. Here, I assume that increments represent one year of growth and therefore age until validation of increment counts can be completed. Fish age was estimated for each spine section by counting the number of translucent zones (Figure 4). Two independent readers evaluated spine increments on a section without knowledge of fish length or date of capture. Spine sections for which reader disagreement occurred were reevaluated simultaneously by both readers and a consensus count was recorded as the final age estimate in whole years. If a consensus could not be reached, then the spine section was discarded from any further analyses. Spine section margins were evaluated as either containing the final translucent zone along the edge or containing the final opaque zone. Using age-3, -4, and -5 fish, timing of increment formation was estimated by examining the monthly proportion of spines with translucent zones at the spine edge. Fractional age estimates were calculated based on the date of capture and the presumed birth date of 1 July which took into account when translucent zones were deposited and when spawning season peaked.

Between-reader precision was examined in two ways: Age estimates were compared between readers to determine the percentage of age estimates that agreed exactly and agreed within one year. Additionally, average percent error (APE) between

ages assigned by readers was computed using the following equation (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 is the number of aged samples, R is the number of times a fish was aged, X_{ij} is the ith age determination of the jth fish, and X_j is the average age calculated for the jth fish.

To evaluate growth, observed individual lengths-at-age were fitted to the von Bertalanffy growth equation (von Bertalanffy 1938) applied separately to males and females:

$$FL_t = FL_\infty[1 - e^{-k(t-t_0)}]$$

where FL_t is the FL at age t, FL_∞ is the asymptotic length, k is the von Bertalanffy growth coefficient; and t_0 is the hypothetical age at a FL of zero.

Reproduction.—Gonads were removed from each fish, and the posterior portion of each gonad was fixed in 11% seawater-buffered formalin for up to two weeks, then transferred to 50% isopropanol. Gonad samples were processed using standard histological procedures (Harris and McGovern 1997, Wyanski *et al.* 2000, Harris *et al.* 2004). The tissue samples were vacuum-infiltrated and blocked in paraffin wax. Three transverse sections (~7 µm thick) were cut using a rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counter-stained with eosin-y.

Stained sections were viewed under a compound microscope to determine sex and reproductive state for samples from 1991 and 1998-1999 by MARMAP personnel, 1992-

1997 by Moore (2001), and 2000-2012 by readers in the current study in that sex and reproductive state were assessed according to a modified version of the histological criteria utilized in previous reef fish studies with slight modification in terminology (Table 1; Brown-Peterson *et al.* 2011). For each histological slide throughout all years, two readers independently assigned sex and reproductive state without knowledge of date of capture, specimen length, or specimen age. If differences in maturity assignments occurred, readers examined the slide simultaneously to agree to a consensus assignment. If no consensus decision was reached, that specimen was eliminated from the analyses. To examine male reproductive morphology in Gray Triggerfish, specimens were sectioned lengthwise to examine the structure of testes and associated ducts. Lengthwise sections were stained and mounted using the same techniques previously described.

The gonads of male Triggerfishes are unique in their structure and function. In general for many fish species, the shapes of the male and female gonads are similar, in that they consist of two lobes that are posteriorly attached and release the gametes via oviduct (female) or spermatic duct (male). Female Triggerfish gonads are similar in shape compared to other fish species, containing two lobes that are posteriorly attached and release the eggs via the oviduct (Figure 5a). Comparatively, male gonads of Triggerfishes consist of testes, spermatic duct, and accessory glands (Figure 5b). The accessory glands are used to store spermatozoa before spawning. Both the testes and accessory glands are needed in order to assign the most accurate reproductive state to males (Kelly *et al.* in prep).

To ensure that immature and resting specimens were assigned correctly, the size frequency of fish that were definitely mature (i.e., developing, spawning, or regressing)

was compared to size frequencies of immature and regenerating fish. Fish of uncertain sex and maturity were excluded from this data analysis. If little or no overlap was identified in FL of immature and regenerating specimens, it was assumed that the stages were assessed correctly.

Spawning activity in females was denoted by the presence of late-developing oocytes and postovulatory complexes (POCs). Spawning season was defined as the date when POCs first appeared in a specimen until the latest date when POCs appeared in a specimen. Stages of POCs were assigned according to the level of degeneration present in the follicle according to Moore (2001). Stage-1 POCs showed little degeneration, large size, distinct thecal and granulose layers, and a highly convoluted lumen. Stage-2 POCs were smaller than stage-1 POCs, showed degeneration of the granulose and thecal layers, and a less distinct lumen compared to stage-1 POCs. Stage-3 POCs showed a characteristic triangular shape of the granulose layer, an often indistinct thecal layer, and a reduced or absent lumen. To determine spawning frequency, the methods in Hunter *et al.* (1986) were used to calculate overall counts of active, non-spawning females (i.e., yolked oocytes and stage-1 POCs) and spawning females (i.e., stage-2 and stage-3 POCs which represent approximately 24 hours following Hunter *et al.* 1986) by month and in the peak spawning season. The proportion of spawning females was calculated for each month during the spawning season by dividing the total number of active, non-spawning females and spawning females by the total number of spawning females. The proportion of spawning females in the peak spawning season was then multiplied by the number of days in the peak spawning season. It was assumed that all mature females participate in reproduction throughout the spawning season.

Statistical analysis.—In order to determine if the population size structure of Gray Triggerfish changed between the two time periods, I utilized two separate Kolmogorov-Smirnov (K-S) tests (one for males and one for females) to test the null hypothesis that size structure of fish collected from 1994-1997 did not differ from 2009-2012. I also tested for differences in the size frequency distribution between males and females for the full time series (1991-2012) and within each time period (1994-1997 and 2009-2012) using K-S tests. A Student's t-test was used to test for differences in mean lengths between males and females for the full time series. Additionally, for the 2009-2012 time period, I compared the age frequency distributions between males and females in order to determine if age structure differed between the sexes.

To determine if significant differences existed in mean size between males and females and between the two time periods, I utilized a two-factor ANOVA with size (FL) as the dependent variable and the time period and sex as the fixed factors. A Student's t-test was used to test for significant differences in mean ages between males and females within the current time period (2009-2012).

Von Bertalanffy growth curves were fit to non-weighted mean observed lengths-at-age for males and females of Gray Triggerfish during 2009-2012 using the von Bertalanffy growth equation (von Bertalanffy 1938). Eight fish caught in *Sargassum* spp. off the South Carolina coast were assumed to be age 0 and used to represent both sexes even though sex was not determined. These fish were included in order to provide a more representative estimate of growth as fish this small are rarely collected in the chevron trap survey due to the location of the gear and mesh size. Growth curve parameters were compared between sexes using a variance ratio test.

Chi-square tests were used to determine if sex ratios differed from 1:1 within the two time periods.

A subset of fish size data were selected in four ways to determine if fish size related to depth of capture. First, in order to control for possible long-term shifts in size structure of the population, I only used data from 2004 to 2012. Second, in order to control for latitudinally-related water temperature trends, I established two main areas of interest: a North Carolina area (NC-Area) from 33-35°N and 76-78°W and a South Carolina area (SC-Area) from 32-33°N and 79-80°W. Third, I selected data from two discrete depth zones: < 36 m and 40-65 m. Last, considering main sampling efforts conducted by SERFS occurred during May to September, only these months were used in the analyses. For each of the areas, I tested the following null hypotheses: 1) male or female sizes did not differ with depth of capture using linear regression analyses and 2) male or female size frequency distributions did not differ between the two depth zones using K-S tests.

Statistical analyses were conducted in SPSS (IBM Corp. 2012) and RStudio (RStudio 2013). The K-S tests, two-factor ANOVA, Student's t-tests, chi-square tests, and linear regression analyses used in the current study followed the methodology of Sokal and Rohlf (1995). The results were considered significant at p-values less than 0.05. If assumptions for statistical tests were not met, then data were log transformed (Appendix Table 1).

RESULTS

Sampling

Gray Triggerfish sampling from 1991-2012 ranged from 34.60°N, 76.19°W to 27.23°N, 80.05°W. A total of 7685 Gray Triggerfish were collected during this period (44% male, 54% female, 3% unknown sex; Table 2) from depths of 14-92 m. The mean size of males (337 mm FL) was significantly larger than females (304 mm FL; $t = -13.46$, $p < 0.0001$) based on the entire dataset. The size frequency analysis indicated a significant difference ($Z = 6.3$, $p < 0.001$; Figure 6).

Mean size of Gray Triggerfish increased significantly from the 1994-1997 time period to the 2009-2012 time period (Two-factor ANOVA: $F = 95.8$, $df = 1$, $p < 0.001$ for sex and $F = 109.8$, $df = 1$, $p < 0.001$ for time period; Appendix Table 2). Size frequencies of males and females were significantly different between the two time periods (Males: $Z = 3.8$, $p < 0.001$; Females: $Z = 3.8$, $p < 0.001$) and shifted to a greater proportion of larger fish for both sexes in 2009-2012 (Figure 7).

Male size increased with depth for fish caught in the NC-Area ($R^2 = 0.49$; $p < 0.001$; Figure 8a) and the SC-Area ($R^2 = 0.24$; $p < 0.001$; Figure 8b). Female size also increased with depth (NC-Area: $R^2 = 0.52$; $p < 0.001$; Figure 8a; SC-Area: $R^2 = 0.34$; $p < 0.001$; Figure 8b). Additionally, the size class distribution of males in both areas indicated a significantly higher proportion of larger males in the deeper zone (NC-Area: $Z = 4.1$; $p < 0.01$; SC-Area: $Z = 4.0$; $p < 0.01$; Figure 9). Females exhibited a similar trend (NC-Area: $Z = 3.8$; $p < 0.01$; SC-Area: $Z = 5.6$; $p < 0.01$; Figure 10).

Age and Growth

Of the 1372 Gray Triggerfish caught from 2009 to 2012, ages were determined for 1261 fish (92%). The remaining specimens were unused due to missing, broken, or unreadable spines. Agreement between readers occurred for 43% of the spine sections, and age estimates were within one year of each other for an additional 32%. The APE for the current study was 12.5%, and the associated coefficient of variation was 1.3. The percent of spines with translucent edges was around 50% from April to August then dropped to less than 30% for September to October (Figure 11). Age frequency distributions between males and females were similar (K-S test: $Z = 0.8$, $p = 0.575$; Figure 12). However, mean ages differed between males and females (Student's t-test: $t = -1.97$, $p = 0.02$; Table 2).

Von Bertalanffy growth models differed between males and females (variance ratio test: $F = 52.71$, $p < 0.001$; Table 3); thus sex-specific growth curves were fitted to the growth model, yielding to the following estimates of the von Bertalanffy parameters (Figure 13 and Table 3):

$$FL_t = 412[1- e^{-0.63(t+0.36)}] \text{ for males}$$

$$FL_t = 351[1- e^{-0.98(t+0.17)}] \text{ for females}$$

Reproduction

A total of 7644 gonads were collected during 1991-2012. Sex and reproductive state were assigned to 6894 (90%). These gonad samples were examined and analyzed by various readers throughout 1991-2012 as previously mentioned (1991 and 1998-1999 by

MARMAP personnel, 1992-1997 by Moore (2001), and 2000-2012 by readers in the current study).

For 1991-2012, immature Gray Triggerfish made up 3% (n = 217) of the specimens for which reproductive state was determined. Correct assignment of reproductive tissue to immature and regenerating gonad categories was indicated by the complete or near-complete overlap in the left tail of the size frequencies for definitely mature (i.e., developing, spawning, and regressing states) and regenerating-state specimens and by the separation in the size frequencies for immature and regenerating-state specimens (Figure 14).

In order to determine female spawning season, fish throughout all years (1991-2012) were examined due to low sample sizes of spawning indicator females during the two time periods (95 in 1994-1997 and 6 in 2009-2012 compared to 176 in 1991-2012).

Based on the entire dataset from 1991 to 2012, the beginning of the spawning season was April 30, which was the earliest date of POCs occurring in females observed in any year. The end of the spawning season was September 29, which was the latest date of POCs occurring in females observed in any year. Note that only one spawning female was captured in April (adult fish = 71), three captured in September (adult fish = 1295), and zero captured in October (adult fish = 40) from 1991-2012. In addition, no spawning females were captured after August 28th for that month. Therefore, a more conservative estimate of May 5-August 28 for the beginning and end dates for the spawning season were used, resulting in a spawning season of 115 days (Figure 15).

Among females with vitellogenic oocytes, the proportion of females with stage-2 and -3 POCs ranged from 0.03 in May to 0.20 in April (Table 4). The proportion of

spawning females was 0.01 during the peak spawning season (May to August). The spawning periodicity (1/0.01) was approximately every 10 days. With a spawning season of approximately 115 days in the U.S. South Atlantic (May 5th to August 28th), a female can potentially spawn approximately 12 times.

For the remaining analyses, both time periods were used to determine if any shifts occurred in sex ratios by FL and length at 50% maturity. Age at 50% maturity was estimated only for 2009-2012, as ages were only available for that time period. A total of 4000 gonad samples were collected during the two time periods (2633 in 1994-1998; 1367 in 2009-2012). Sex and reproductive state were assigned to 3700 (93%) of these fish.

The overall male:female sex ratio for Gray Triggerfish collected during 1994-1997 was 1:1.19 and differed significantly from a 1:1 ratio (Table 5). Females that were 350 mm FL or smaller were more abundant than males, and the sex ratio significantly differed from 1:1 for females 151-350 mm FL. Males larger than 401 mm FL were more abundant than females, and the sex ratio significantly differed from 1:1 for males 401-500 mm FL. Sample sizes of males and females that were 501-600 mm FL were low (i.e., less than 10 fish) and therefore, chi-square analyses were not performed for these size classes.

The overall male:female sex ratio for Gray Triggerfish collected during 2009-2012 was 1:1.34 and differed significantly from a 1:1 ratio (Table 5). Similar results were found in the current population compared to 1994-1997. Females that were 350 mm FL or smaller were more abundant than males of the same size, and the sex ratio significantly differed from 1:1 for 251-350 mm FL. However, males were more abundant at the

smallest size class of 151-200 mm FL and the sex ratio did not significantly differ. Males larger than 401 mm FL were more abundant than females, and the sex ratio differed significantly from 1:1 for males 401-500 mm FL. Sample sizes of males and females that were 501-550 mm FL were low (i.e., less than 10 fish) and therefore, a chi-square analysis was not performed for this size class. For both time periods, females that were 351-400 mm FL were more abundant than males of the same size; however, the sex ratio did not differ significantly from 1:1 in this size class.

Females were more abundant than males for most ages except ages 0, 7, and 10 (Table 6). The sex ratio significantly differed from 1:1 for ages 2-4 and did not differ significantly for ages 1 and 5-8. For ages 0, 9, and 10, chi-square analyses were not performed due to low sample sizes.

Based on the data by Moore (2001) for histological samples collected from 1994 to 1997, the smallest mature male was 165 mm FL and the largest immature male was 265 mm FL. Male size at 50% maturity was 184 mm FL (95% confidence interval (CI) = 175-191 mm), and all males larger than 271-280 mm FL were mature. The smallest mature female was 152 mm FL and the largest immature female was 297 mm FL. Female size at 50% maturity was 177 mm FL (95% CI = 167-184 mm), and all females larger than 251-260 mm FL were mature with the exception of the largest immature female recorded at 297 mm FL which was 54 mm larger than the next largest immature female at 243 mm FL.

For 2009-2012, the smallest mature male was 183 mm FL, and the youngest was age 0; the largest immature male was 268 mm FL, and the oldest was age 2. Male size at 50% maturity was 174 mm FL (95% CI = 95-205 mm), and all males larger than 281-290

mm FL and 3 years or older were mature. The smallest mature female was 179 mm FL, and the youngest was 0 years; the largest immature female was 388 mm FL, and the oldest was 4 years. Female size at 50% maturity was 196 mm FL (95% CI = 166-214 mm), and all females were mature by 301-310 mm FL with the exception of the largest immature female recorded at 388 mm FL which was 98 mm larger than the next largest immature female at 290 mm FL. All females were mature by 5 years. Model estimated age at 50% maturity for females was -0.38 years and for males was -0.21 years.

DISCUSSION

Population size structure, age, and growth

Mean lengths of males were significantly larger than females. Similar findings have been reported for Gray Triggerfish in the Gulf of Mexico (Hood and Johnson 1997, Ingram 2001). Males and females also exhibited different growth rate parameters in our study with males attaining a larger asymptotic length than females. Ingram (2001) documented a similar trend with fish collected from Alabama. To some degree, this may be related to the sexual dimorphic growth and nesting behaviors documented for this species. Simmons and Szedlemayer (2012) studied the reproductive behavior of Gray Triggerfish utilizing artificial reef habitats in the northern Gulf of Mexico. They documented that a large dominant male patrols a nesting territory, builds, and maintains multiple nests within the territory, and continues to guard the nesting area after fertilization. The larger size of male Gray Triggerfish could be advantageous, allowing them to more adequately defend the nests in order to optimize survival of the eggs.

Increase in the mean size of males and females (Figure 7) occurred in a time that saw an increase in fishing pressure across the region (Figure 1). Fish stocks experiencing overfishing usually exhibit the opposite of this trend. For example, Speckled Hind *Epinephelus drummondhayi* in the same area of the current study is considered an overfished species (National Marine Fisheries Service 2012). Ziskin *et al.* (2011) documented a decrease in average size of Speckled Hind caught in 2004-2007 when compared to historic data from 1979-1981. Similarly, the population of Scamp *Mycteroperca phenax* in the southeastern U.S. Atlantic waters experienced a decrease in size when recent data were compared to historical data (Harris *et al.* 2002). A decline in mean sizes has also been reported for several species of Porgy and Snapper after 15 years of intense fishing pressure in waters offshore of North Carolina (Parker and Dixon 1998).

As seen with other fish species, high fishing pressure has the potential to remove the larger and faster-growing individuals from the population. However, for Gray Triggerfish, high fishing pressure may not have been applied to the population long enough to see a decline in the larger, faster-growing individuals. The increase in mean sizes and the increase in the proportion of larger individuals within the population of Gray Triggerfish may partially be explained by a corresponding decline in the numbers of co-occurring reef fish species that may compete with Gray Triggerfish for resources such as food and shelter. Adult Gray Triggerfish are generalist feeders (Blitch 2000, S. Goldman, unpublished data). They consume a wide range of invertebrate prey items including sponges, crustaceans (such as barnacles and crabs), echinoderms (such as sea urchins, sand dollars, and sea stars), and molluscs (such as bivalves, gastropods, and cephalopods). Many of the declining reef fish species are narrower in their diet (Tremain

and Adams 2012). As co-occurring species such as Scamp, Red Porgy *Pagrus pagrus*, and Speckled Hind have declined in numbers, Gray Triggerfish may have experienced an increase in the availability of food items which could lead to increased growth rates and ultimately a shift in the proportion of larger individuals within the population.

The first dorsal spine has been the accepted aging structure for Triggerfish species for over 40 years (Ofori-Danson 1989, Ingram 2001, Moore 2001, Bernardes 2002, Aggrey-Fynn 2009, Fioramonti 2012). The age range of Gray Triggerfish from this study is similar to other reports of age for Gray Triggerfish (Johnson and Saloman 1984, Hood and Johnson 1997, Moore 2001, Bernardes 2002). However, ages based on dorsal spines have yet to be validated (i.e., confirm that structures interpreted as annual growth zones are in fact annual growth zones). Two studies have attempted to validate ages for dorsal spines by chemically-marking laboratory-held Gray Triggerfish collected from the Gulf of Mexico with oxytetracycline (Hood and Johnson 1997, Fioramonti 2012). Hood and Johnson (1997) chemically-marked 12 fish and then held them for one year before sacrificing them and processing the fish for age determination. In all 12 fish, the chemical mark was still on the edge of the spine indicating that no additional growth occurred on the spine during the year in captivity. In contrast, Fioramonti (2012) chemically marked four Gray Triggerfish. After eight months, fish were sacrificed and processed for age determination. Fioramonti (2012) reported that one translucent zone formed on the spines of the four fish beyond the chemical mark. Due to the conflicting results of these two studies, spines have yet to be truly validated as an appropriate aging structure for this species. Additionally, preliminary results from an ongoing study indicate that sectioned sagittal otoliths and vertebrae may provide different age estimates when compared to the

first dorsal spine sections of Gray Triggerfish (V. Shervette, unpublished data). The first dorsal spine was used in the current study because it is the accepted aging structure for Gray Triggerfish in the literature, allowing the findings in the current study to be compared to other literature which used the first dorsal spine to age Gray Triggerfish.

Precision is another important consideration in the selection and use of an aging structure (Campana 2001) and Gray Triggerfish dorsal spine precision is relatively low compared to the precision reported for other species when otoliths are used. Studies that utilize sectioned otoliths for aging reef fish species typically report APE values of < 5% which indicates high precision (Lombardi-Carlson *et al.* 2008, Collier and Stewart 2010). The current study and Fioramonti (2012) reported much higher APE values for Gray Triggerfish dorsal spines (> 10%). Gray Triggerfish dorsal spine shapes and sizes are more variable compared to many otoliths (Figure 16). To improve precision in the current study, criteria were created and strictly followed by each reader. Thus far, the ages reported in this study are the best available estimate for age and growth for this population, assuming that increments represent one year of growth. However, whether the ages used in this study represent the true age of this species is still unknown so caution must be used when interpreting these data.

The results from our monthly proportion of translucent edges indicate that annulus formation may occur in the late spring to early summer, which is consistent with findings by Moore (2001). In that study, mean marginal increments were plotted by month for ages 3-7 Gray Triggerfish from fishery-independent and -dependent samples from U.S. Atlantic waters. Moore (2001) concluded that increment formation occurred in June. Johnson and Saloman (1984) reported increment formation for the Gulf of Mexico

Gray Triggerfish in April-August. Other reef fish also exhibit summer increment formation (Ziskin *et al.* 2011, Wyanski *et al.* 2000, Garcia *et al.* 2003). For example, Snowy Grouper *Epinephelus niveatus* in North Carolina and South Carolina offshore waters form annuli from April to May (Wyanski *et al.* 2000). Yellowtail Snapper *Ocyurus chrysurus* in Florida form annuli in March-April (Garcia *et al.* 2003). Speckled Hind from Atlantic waters form annuli in June-August (Ziskin *et al.* 2011). The formation of the translucent zone during spring months would be expected considering translucent zones are indicative of slower somatic growth occurring for that fish during that time. Considering Gray Triggerfish generally spawn in the summer months (May-August), they are investing more energy into growth of their gametes to optimize survival of their offspring compared to the less energy they are investing in their somatic growth.

In the current study, fish sampled in 2009-2012 ranged in age from 0-10 for males and 0-9 for females. Fioramonti (2012) reported similar maximum ages for males (8) and females (9). Johnson and Saloman (1984) reported that males lived up to 13 years and females up to 12. Previously, Gray Triggerfish have been reported to grow rapidly and obtain a relatively large size by the end of the first year (Hood and Johnson 1997, Ingram 2001, Fioramonti 2012), which is consistent with the current study.

The younger age classes (i.e., ages 0) were observed infrequently for both sexes in the current study. The low sample sizes of age-0 fish collected in this study could be influenced by the fact that the early life stages of Gray Triggerfish associate with *Sargassum* spp. and are not available to bottom gears similar to that used in the current study. The exact age when larval and juvenile Gray Triggerfish cease to associate with *Sargassum* spp. and establish in reef habitats is unknown. Another possible factor

influencing the low sample sizes of smaller and younger Gray Triggerfish in this study is the abundance of predators in the chevron traps. Smaller fish may exhibit predator avoidance if larger predators are inside the traps.

Reproduction

Some of the data from 1992-1997 reported in the current study came from the fishery-independent samples examined and analyzed in Moore's (2001) study of Gray Triggerfish reproductive biology. The current study expanded on the original reproductive information from Moore (2001) and incorporates an additional 15 years of data. Gray Triggerfish from the southeastern U.S. Atlantic waters spawned from April to September, which is similar to the spawning season reported previously for the southeastern U.S. Atlantic coast (Moore 2001) and the Gulf of Mexico (Hood and Johnson 1997, Ingram 2001). Based on histological analyses of gonadal tissue, Gray Triggerfish is a gonochoristic species, and females are group synchronous, indeterminant, batch spawners. Spawning appears to start as early as April based on a few spawning-capable females and spawning continues through September. The peak spawning period is May-August for fish from the current study.

Based on data from the current study, females could spawn 12 times throughout the spawning season, which is more frequent than the spawning frequency reported previously for the southeastern U.S. Atlantic coast (Moore 2001). Considering the expansion of the current study from Moore (2001), the sample size of spawning females has increased resulting in a more accurate female spawning frequency for Gray Triggerfish off the southeastern U.S. Atlantic coast. Spawning frequency of Gray

Triggerfish in the Gulf of Mexico was reported to be two times more frequent than that of the current study (Ingram 2001). Ingram (2001) used oocytes in the final maturation stage (i.e., mid-late developing oocytes) to determine spawning frequency which contrasts with the current study and Moore (2001) in which the late-developing and three stages of the post-ovulatory complexes were used as spawning indicators. It is unclear as to why Ingram (2001) did not use post-ovulatory complexes to also represent spawning indicator females. Perhaps sample sizes of females collected with post-ovulatory complexes were too low in the study by Ingram (2001). Considering post-ovulatory complexes indicate that the female has recently spawned her oocytes, these reproductive stages are essential in determining the accurate spawning season, periodicity, and frequency for female Gray Triggerfish.

Length at which 50% of the population has reached sexual maturity was similar between males and females in the current study. Sizes at maturity are similar to sizes reported previously for Gray Triggerfish off the southeastern U.S. Atlantic coast (Moore 2001) and in the Gulf of Mexico (Hood and Johnson 1997, Ingram 2001).

Age at 50% maturity for the current study indicates that a large proportion of the population reached sexual maturity by age 1. Considering the discrete spawning season of Gray Triggerfish, this means that most fish born the previous year are sexually mature by the start of the next spawning season and could contribute to the reproductive output of the population at one year of age. As aging criteria were created and applied for the current study, comparing ages among different studies of Gray Triggerfish is problematic considering each study had a certain aging protocol associated with their spine sections.

Male gonads of triggerfishes are unique in their structure and function in that they consist of testes, spermatic duct, and accessory glands (Figure 5b). The accessory glands are used to store spermatozoa before spawning. The purpose of storing spermatozoa in the accessory glands could be related to the reproductive behavior of this fish species compared to other reef fish species. Male Gray Triggerfish have been observed to build several nests into the sediment, and females will lay their eggs in those nests (Simmons and Szedlemayer 2012). Considering the number of nests and the distance between each nest, the storage of spermatozoa in the male accessory glands would be necessary in order to assure fertilization of eggs in each nest.

Conclusion

The results from the current study provide essential life history information for any future stock assessments of the Gray Triggerfish population off the southeastern U.S. Atlantic coast. For example, sexes may need to be modeled separately in stock assessments based on the differences in von Bertalanffy growth parameters between males and females. The current study also may provide implications of current fishing practices on the Gray Triggerfish population off the southeastern U.S. Atlantic coast. As fishermen tend to remove larger fish from the population, males may be removed more frequently than females based on the sexually dimorphic growth identified in the current study. This may ultimately affect sex ratios (and may have already been observed in the current study) and the successful formation of spawning harems. Furthermore, as other reef fish species numbers have declined, Gray Triggerfish could be experiencing an increase in the availability of resources such as food items which could lead to increased

growth rates and ultimately a shift in the proportion of larger individuals within the population.

Due to more tightly regulated snapper and grouper fisheries, Gray Triggerfish has become a more targeted and economically valuable fish species in the United States in recent years. However, essential life history information on the Gray Triggerfish population off the southeastern U.S. Atlantic coast is limited; most literature pertains to the Gulf of Mexico Gray Triggerfish population with no peer-reviewed publications available for the Gray Triggerfish population in the U.S. South Atlantic.

The findings from the current study will be incorporated in an upcoming stock assessment. The goal of U.S. fisheries management is to ensure the sustainability of our marine fishery resources and an essential step in that effort includes the Southeast Data, Assessment, and Review (SEDAR). The goal of SEDAR is to improve the scientific quality of stock assessments which emphasizes the use of quantitative data in fisheries management decisions. A successful stock assessment must include a combination of fishery-dependent and -independent information and incorporate the use of high quality life history information for each species. In particular, the current study will provide important fishery-independent life history information in the upcoming benchmark SEDAR 41 stock assessment for South Atlantic Gray Triggerfish and has the potential to affect future management decisions.

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FIGURES

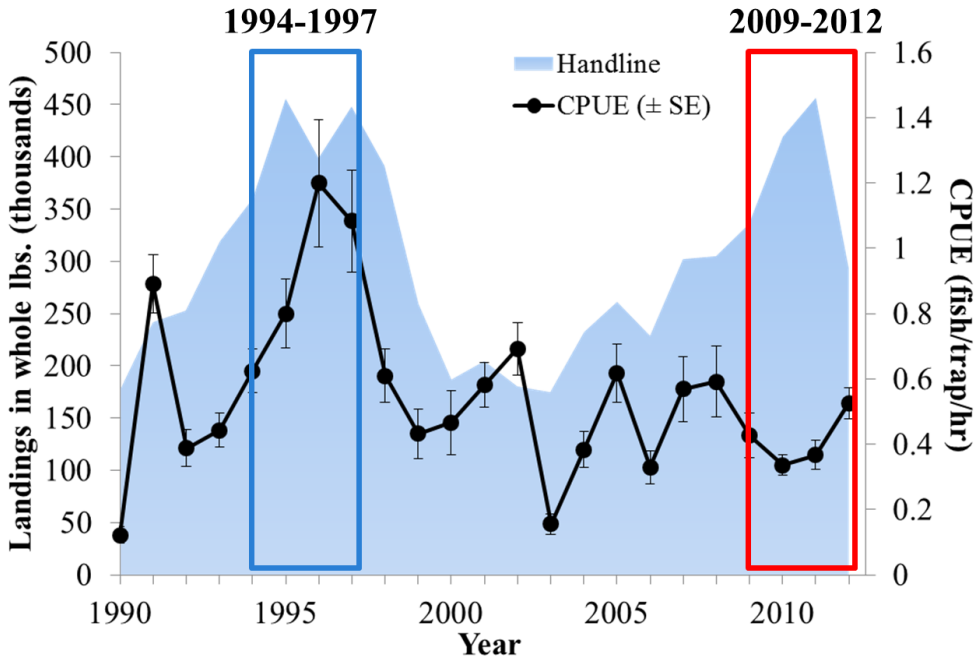


Figure 1.—Commercial landings, represented by handline (the predominant gear for capturing Gray Triggerfish in commercial fisheries) in pounds (thousands) reported by SEDAR (2013) for 1990-2011 and NOAA (2014) for 2012 and chevron trap standardized CPUE (±SE) reported by Ballenger *et al.* (2013) off the southeastern U.S. Atlantic coast from 1990-2012. The two time periods of interest in the current study were overlayed onto the figure to indicate a peak in commercial landings and CPUE from 1994-1997 and peak in commercial landings and relatively low CPUE from 2009-2012. Note: Commercial landings data in pounds (thousands) for 2012 are preliminary (NOAA 2014).

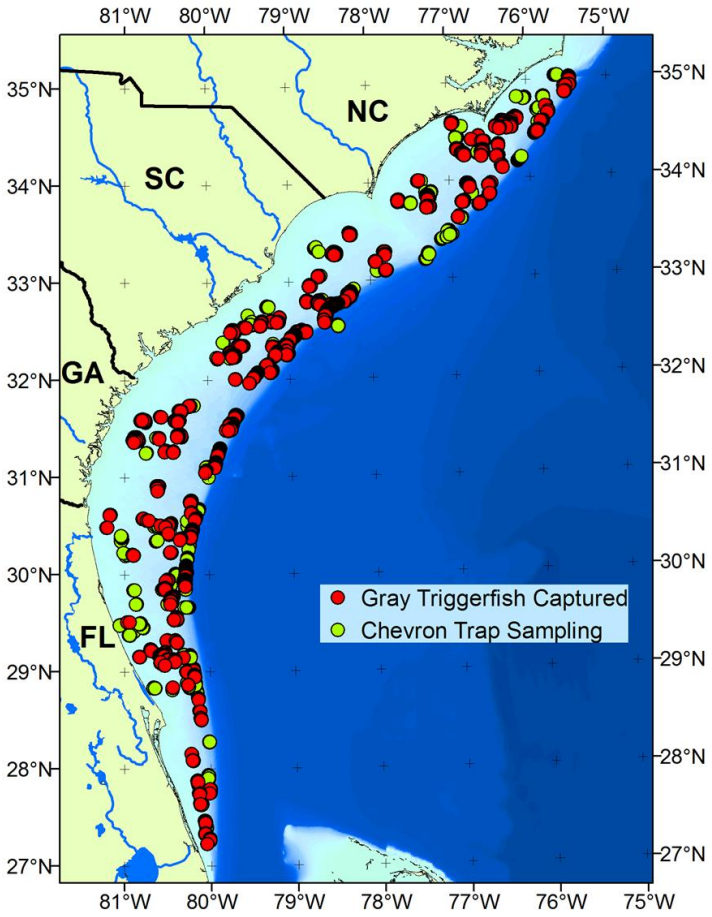


Figure 2.—Chevron trap sampling locations where Gray Triggerfish were captured off the southeastern U.S. Atlantic coast from 1991-2012 by MARMAP (through 2009) and SERFS (2009 to present). Note that symbols may overlap, and sampling coverage in the area varied over the years.

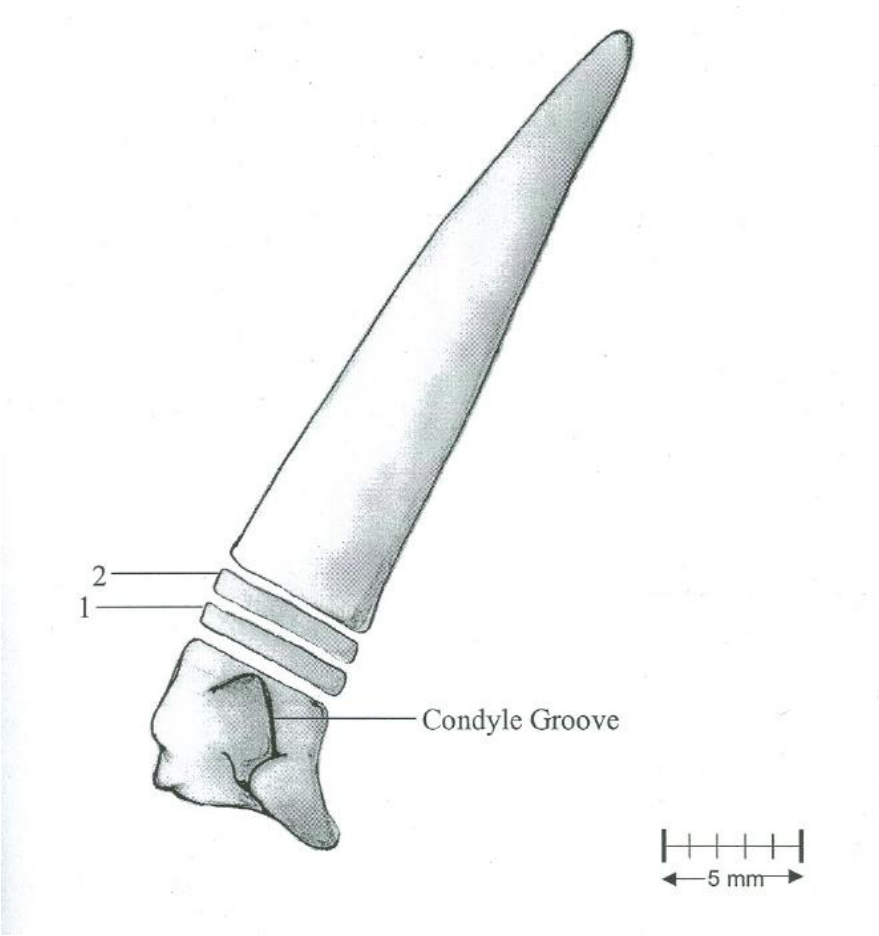


Figure 3.—Lateral view of the Gray Triggerfish spine, showing sections used for age determination are cut basal to the condyle groove.

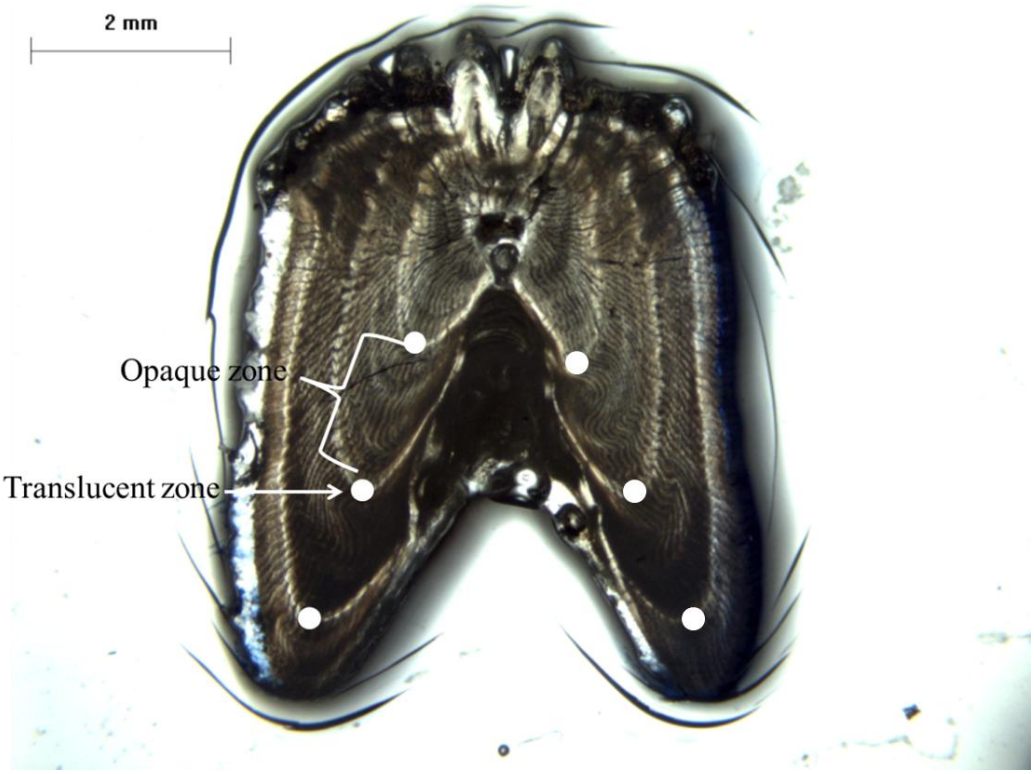
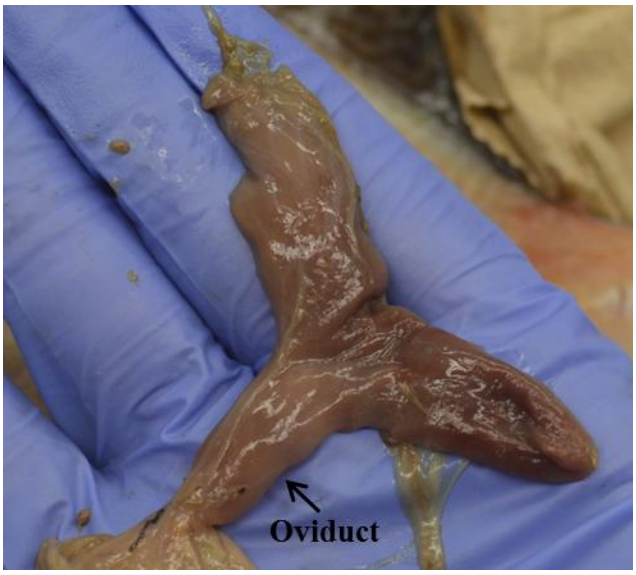


Figure 4.—Cross-section of the Gray Triggerfish spine. An increment is determined by an opaque zone and a translucent zone. For this spine, three translucent zones (dots) were enumerated, resulting in a three-year-old fish.

A



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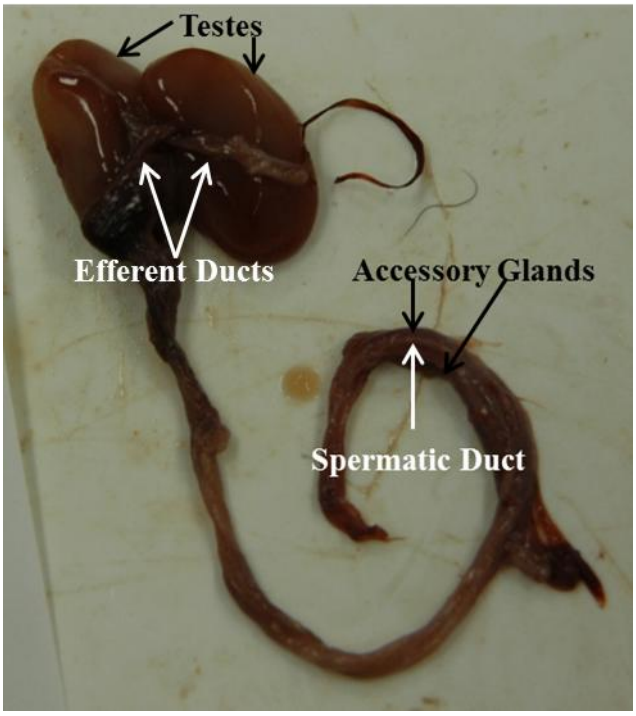

Figure 5.— Gonads of a (A) female Gray Triggerfish with the oviduct as the gamete release pathway and (B) male Queen Triggerfish *Balistes vetula* with the efferent ducts along the testes and the accessory glands surrounding the spermatic duct.

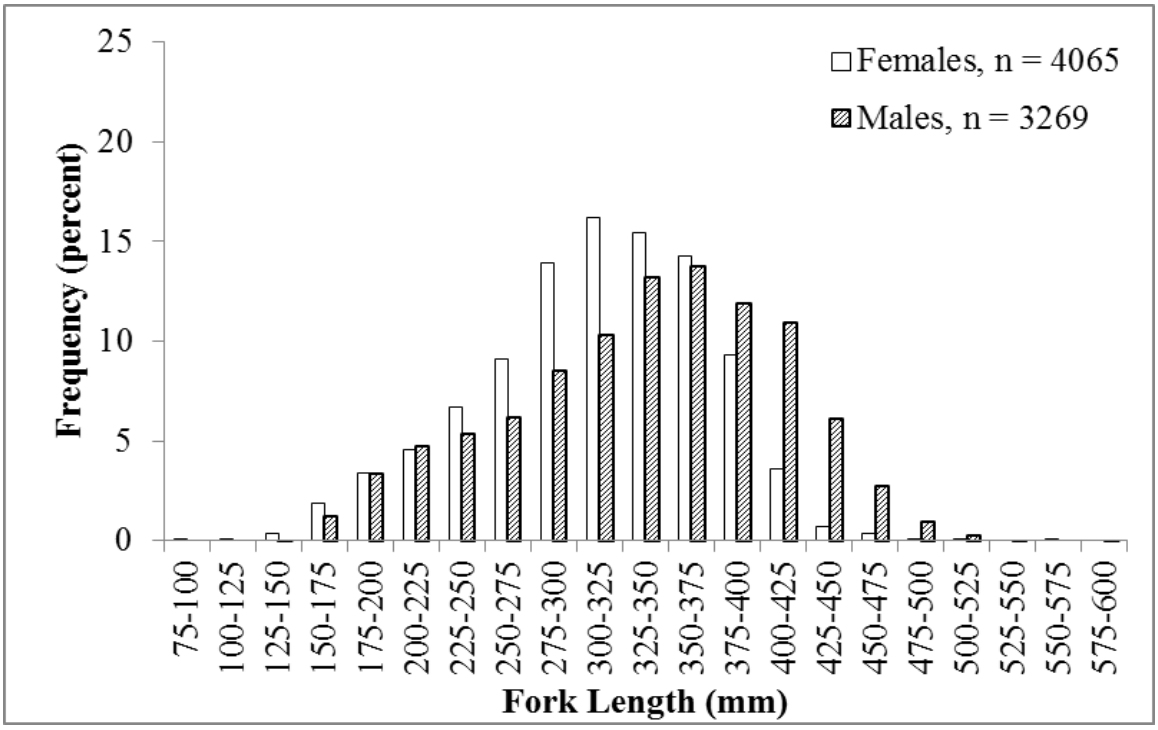

Figure 6.—Size (fork length, mm) frequency distribution for male and female Gray Triggerfish sampled off the southeastern U.S. Altantic coast from 1991-2012 (*n* = number of specimens).

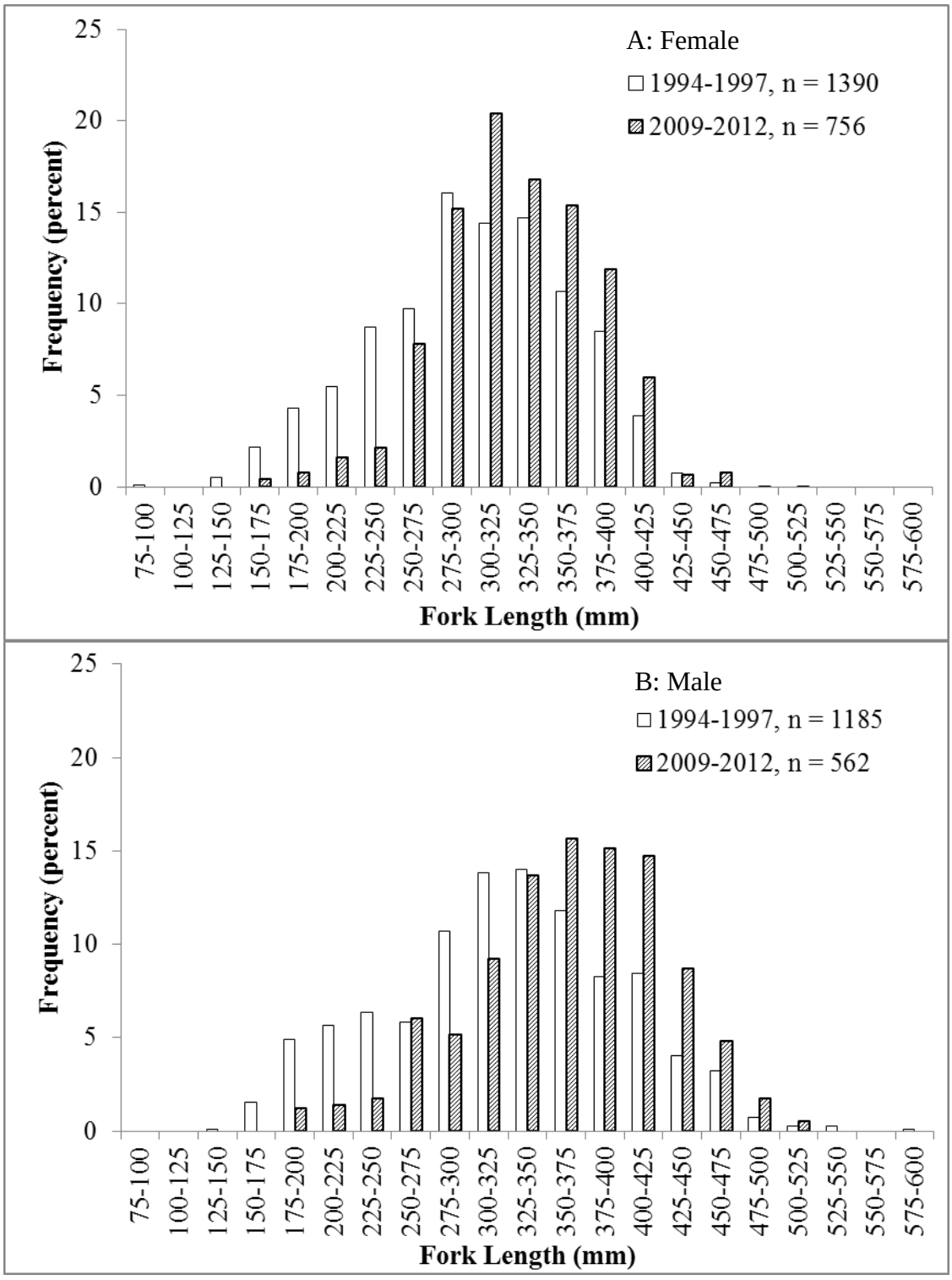

Figure 7.— Size (fork length, mm) frequency distributions of (A) female and (B) male Gray Triggerfish sampled off the southeastern U.S. Atlantic coast during 1994-1997 and 2009-2012 (*n* = number of specimens used in the analysis).

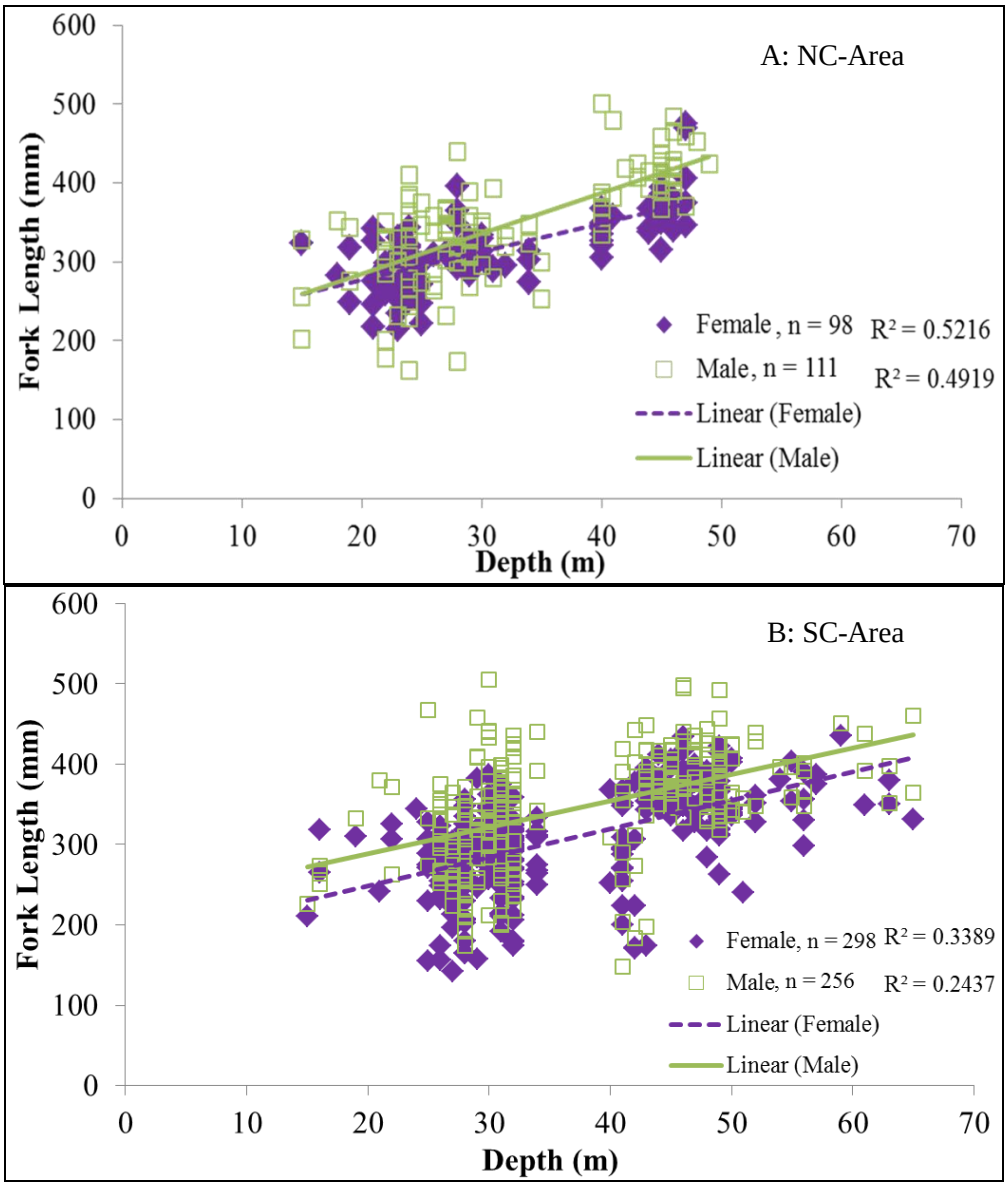

Figure 8.—Size (mm FL) of Gray Triggerfish at depth (m) in (A) NC-Area and (B) SC-Area during 2004-2012 (*n* = number of specimens used in analysis). Linear regression analyses of the slopes were used (NC-Area: R^2 for females = 0.5216 and R^2 for males = 0.4919 and SC-Area: R^2 for females = 0.3389 and R^2 for males = 0.2437).

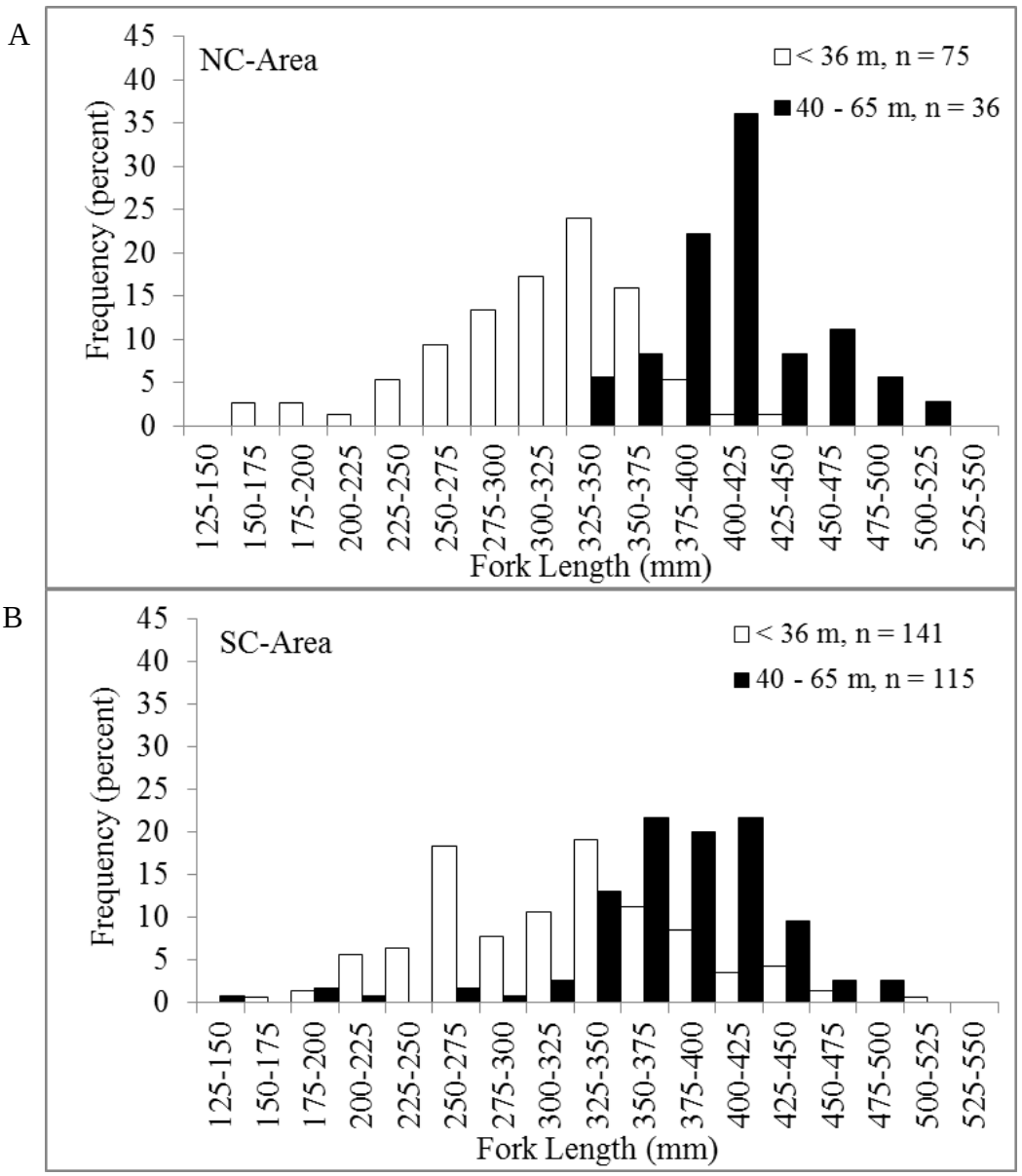

Figure 9.—Size-class (fork length, mm) frequency distributions of male Gray Triggerfish sampled at depths of less than 36 m (open bars) and 40-65 m (closed bars) in the (A) NC-Area and (B) SC-Area during 2004-2012 (*n* = number of chevron traps with Gray Triggerfish present).

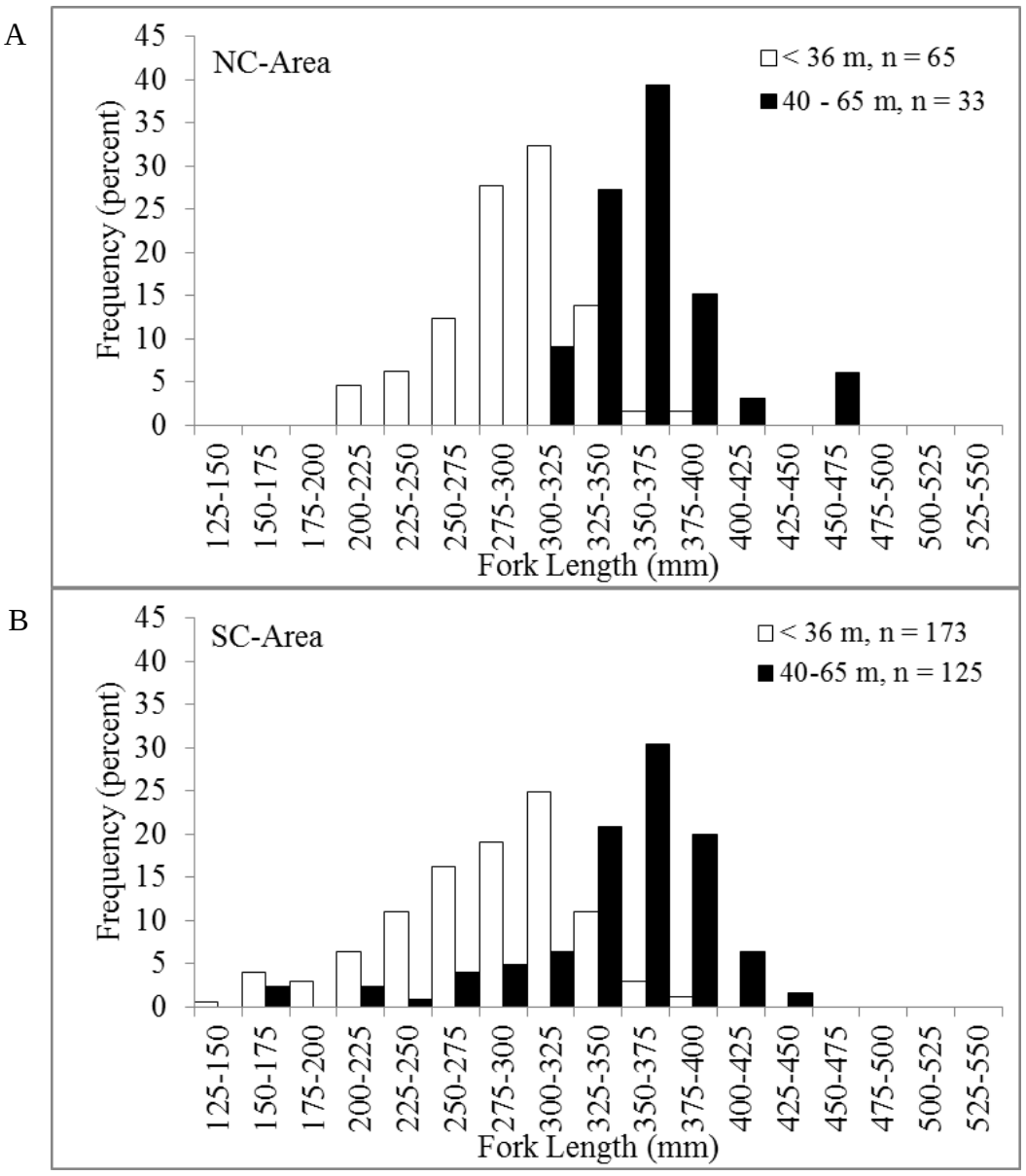

Figure 10.—Size-class (fork length, mm) frequency distributions of female Gray Triggerfish sampled at depths of less than 36 m (open bars) and 40-65 m (closed bars) in the (A) NC-Area and (B) SC-Area during 2004-2012 (*n* = number of chevron traps with Gray Triggerfish present).

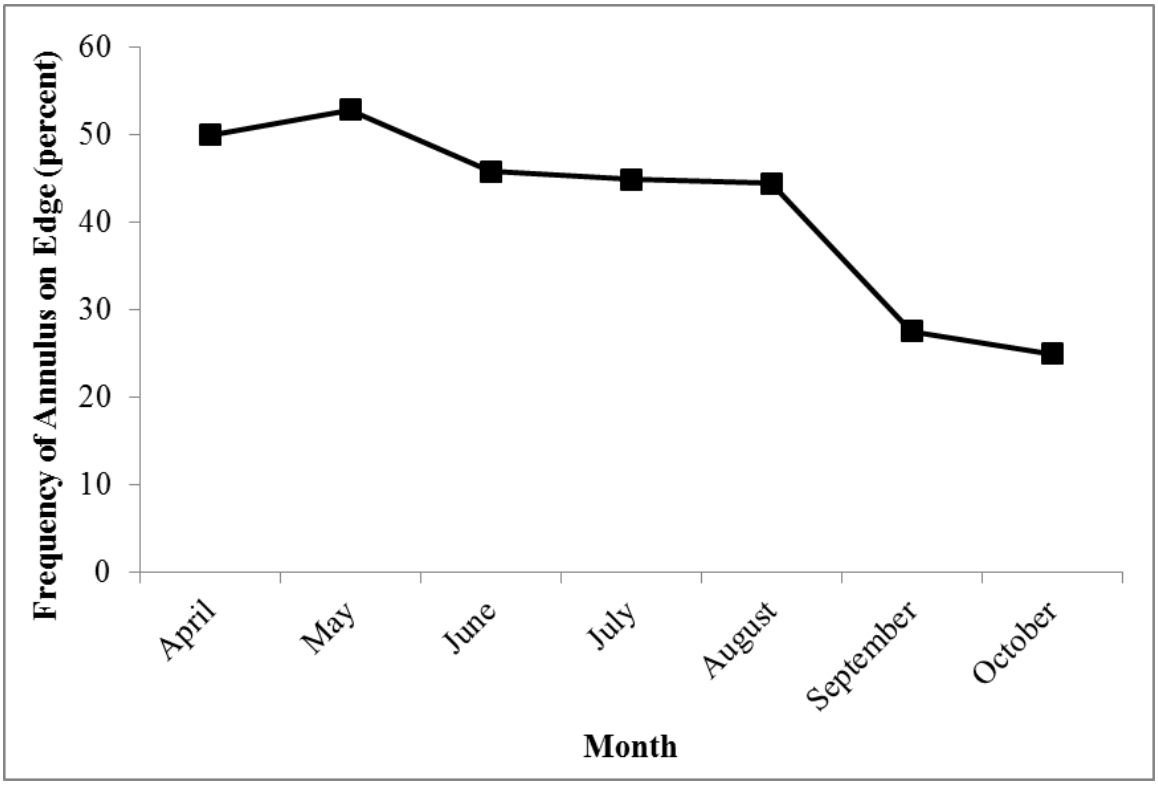

Figure 11.—Percent frequency of annulus (i.e., translucent zone) present on of the first dorsal spines of Gray Triggerfish collected off the southeastern U.S. Atlantic coast edge by month. Edge types were 1) presence of translucent zone on the spine edge and 2) absence of translucent zone on the spine edge.

Figure 12.—Age frequency distribution of female and male Gray Triggerfish sampled off the southeastern U.S. Atlantic coast during 2009-2012 (*n* = number of specimens used in the analysis).

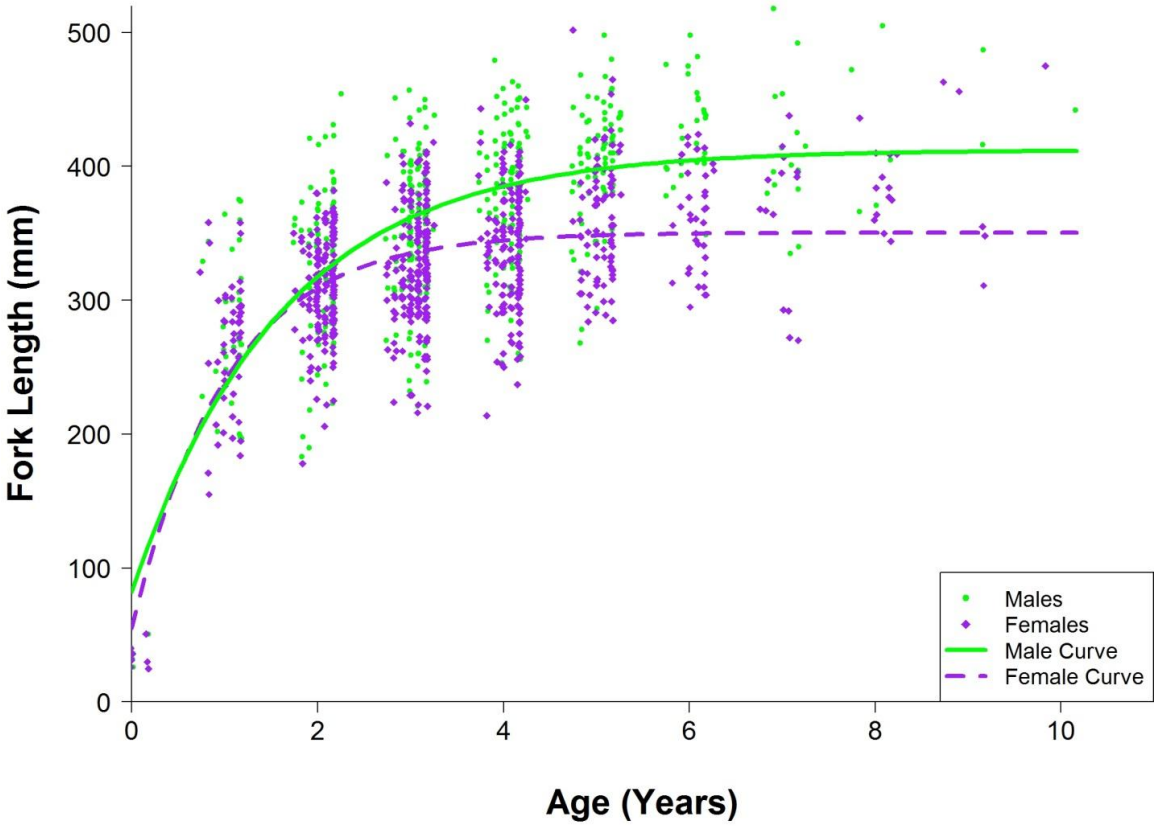

Figure 13.—Fork length at age for male and female Gray Triggerfish collected off the southeastern U.S. Atlantic coast during 2009-2012. Von Bertalanffy growth curves for males (green line) and females (purple line) were fitted to the sex-specific data sets. Eight fish caught in *Sargassum* spp. off the South Carolina coast were assumed to be age 0 and used to represent both sexes even though sex was not determined in order to provide a more representative estimate of von Bertalanffy. These fish give a better estimate of growth within the population off the southeastern U.S. Atlantic coast. See table 3 for von Bertalanffy parameters.

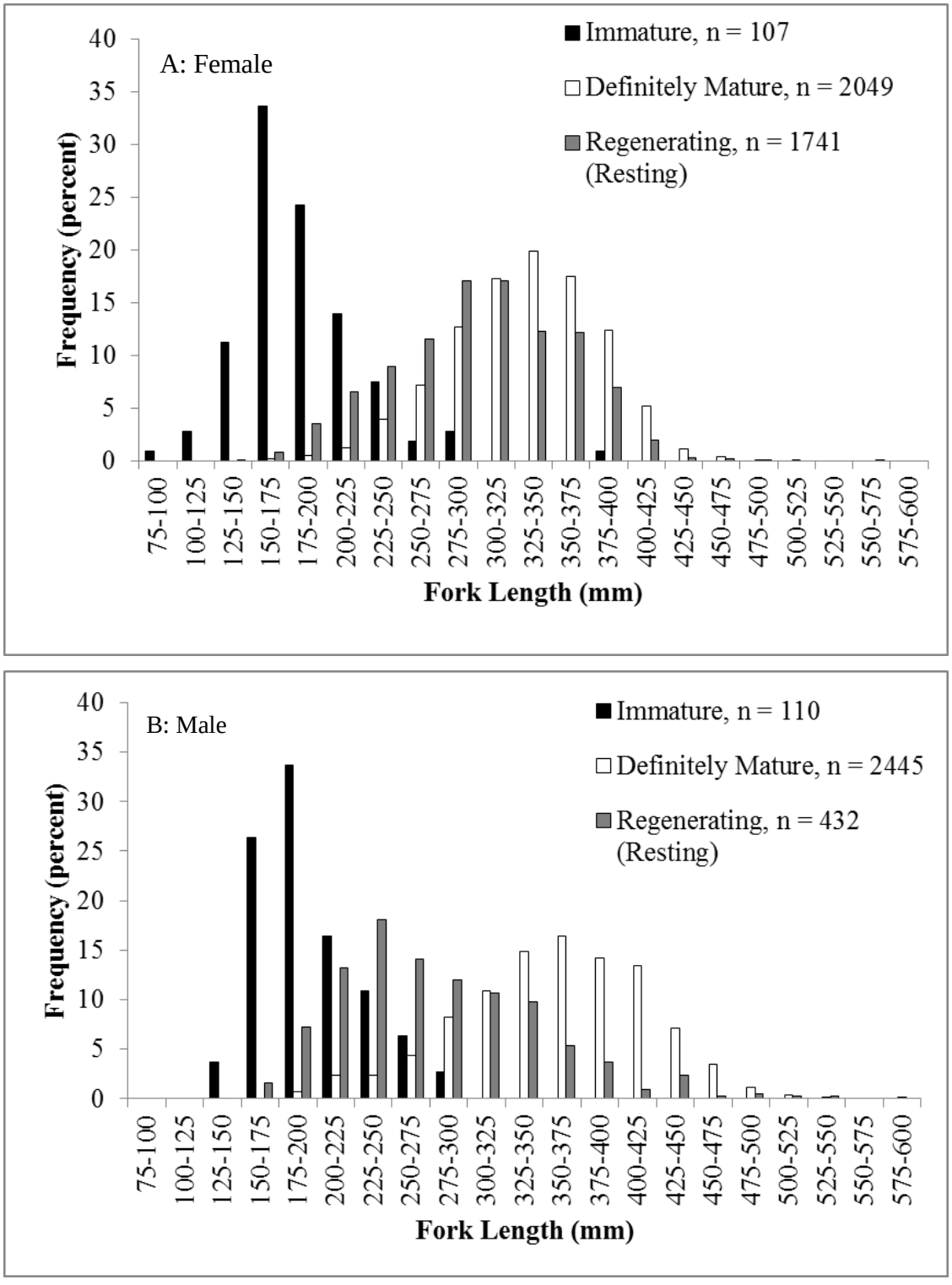

Figure 14.—Size (fork length, mm) frequency distributions of A) female and B) male Gray Triggerfish sampled of different maturity status (gonads categorized as immature, definitely mature [i.e., developing, spawning, or regressing], or regenerating) sampled off the southeastern U.S. Atlantic coast during 1991-2012, which includes data from Moore (2001) (*n* = number of specimens).

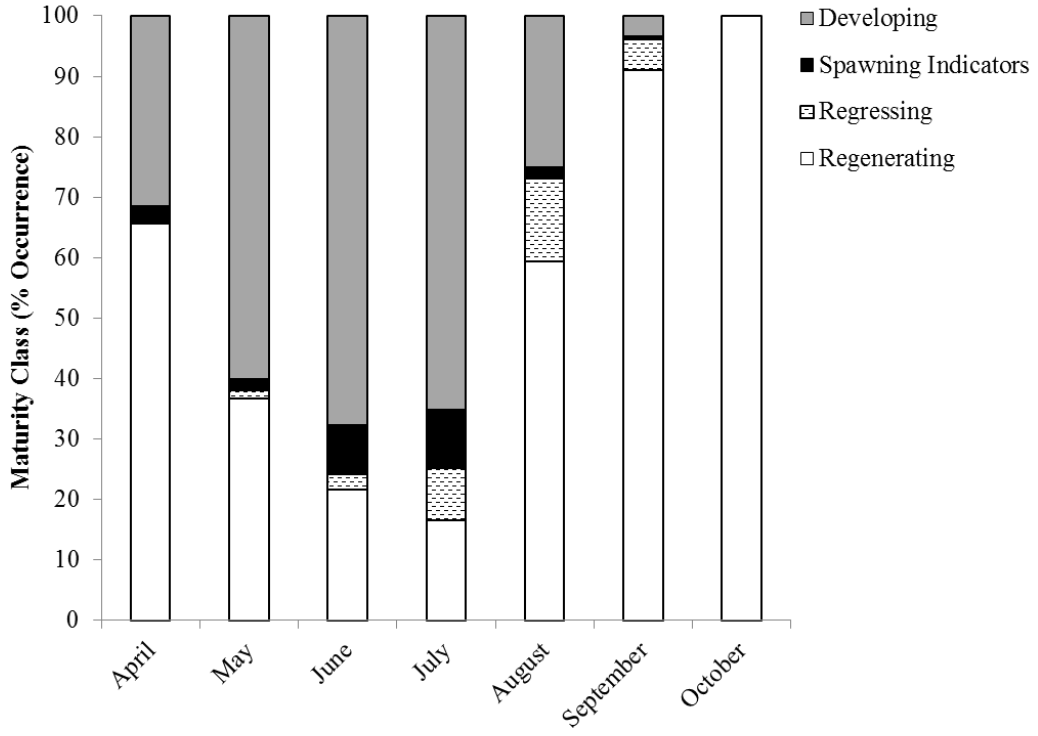

Figure 15.—Reproductive seasonality of female Gray Triggerfish collected during 1991-2012 off the southeastern U.S. Atlantic coast, which includes data from Moore (2001).

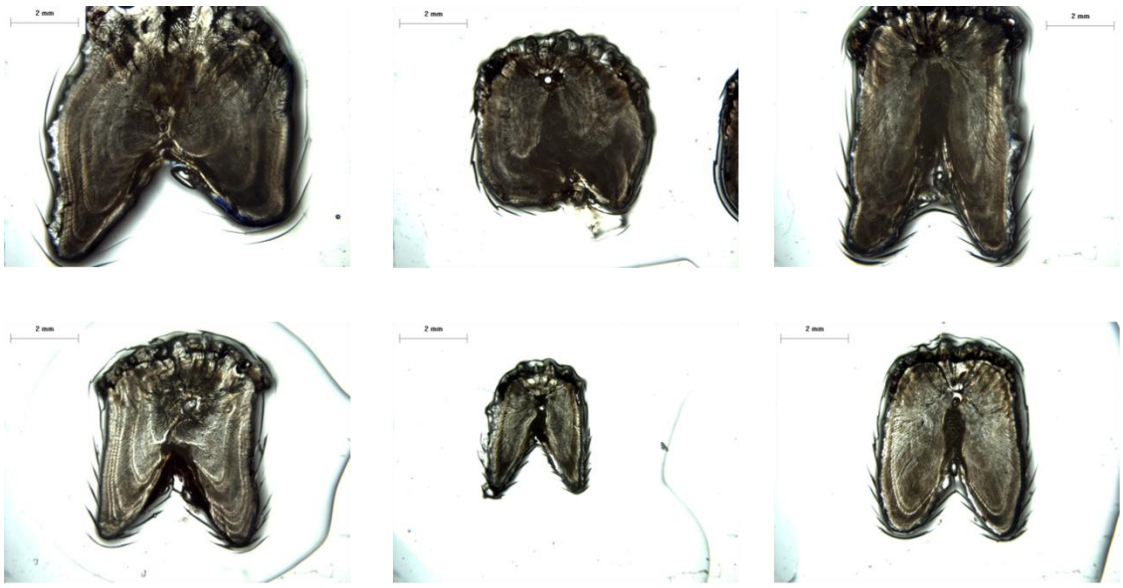

Figure 16.—Images of spine sections, representing variations in shape, size, and quality. See text for details.

TABLES

Table 1.—Histological criteria, modified from Harris and McGovern (1997) and developed by MARMAP to determine reproductive states in various fish species. Terminology in the table has been modified according to Brown-Peterson *et al.* (2011).

Reproductive state	Male	Female
Immature (never spawned)	Small transverse section compared to regenerating male; little or no spermatocyte development	Previtellogenic oocytes only; no evidence of atresia. In comparison with resting female, most previtellogenic oocytes <80 pm. area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, germinal epithelium along margin of lamellae is thicker, ovarian wall is thinner
Developing	Development of cysts containing primary and secondary spermatocytes through some accumulation of spermatozoa in lobular lumina and dorsomedial sinuses	Oocytes undergoing cortical granule (alveoli) formation through nucleus migration and partial coalescence of yolk globules
Spawning capable (formerly "Running ripe")	Predominance of spermatozoa in lobules and dorsomedial sinuses; little or no occurrence of spermatogenesis	Completion of yolk coalescence and hydration in most advanced oocytes. Zona radiata becomes thin. Postovulatory follicles sometimes present
Regressing (formerly "Spent")	No spermatogenesis; some residual spermatozoa in lobules and sinuses	More than 50% of vitellogenic oocytes with alpha- or beta-stage atresia
Regenerating (formerly "Resting")	Little or no spermatocyte development; empty lobules and sinuses	Previtellogenic oocytes only; traces of atresia. In comparison with immature female, most previtellogenic oocytes >80 um, area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongate and convoluted, epithelium along margin of lamellae is thinner, ovarian wall is thicker
Mature specimen, stage unknown	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive stage	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive stage

Table 2.—Overview of geographic, depth (m), size (FL in mm), and age (years) ranges for males and females and over time, including the total number of fish sampled and percentages of males, females, and unknown sex over time.

	1991-2012	**1994-1997**	**2009-2012**
Geographic Range	27.23°N, 80.05°W to 34.60°N, 76.19°W	28.95°N, 80.18°W to 34.59°N, 76.95°W	27.23°N, 80.05°W to 34.59°N, 76.93°W
Depth Range (m)	14-92	15-92	15-87
Total number of fish	7685	2647	1372
% Male	43	45	41
% Female	54	53	56
% Unknown	3	2	3
Size Range (mean)	82-578 (321)	82-578 (314)	155-523 (346)
Male (mean)	136-578 (337)	137-578 (328)	183-523 (367)
Female (mean)	82-560 (304)	82-474 (296)	155-502 (326)
Age Range (mean)	-	-	0-10.2 (3.6)
Male (mean)	-	-	0-10.2 (3.7)
Female (mean)	-	-	0.1-9.8 (3.4)

Table 3.—Sex-specific von Bertalanffy parameters (L_∞ = asymptotic length; k = growth coefficient; t_0 = hypothetical age at zero length) derived from von Bertalanffy growth equations fitted to individual length-at-age data for Gray Triggerfish sampled off the southeastern U.S. Atlantic coast, 2009-2012.

Sex	L_∞	k	t_0
Male	412	0.63	-0.36
Female	351	0.98	-0.17

Table 4.—Female Gray Triggerfish spawning frequency based on histological data from 1991-2012. Spawners had medium or old postovulatory complexes (POCs). Non-spawners are reproductively active (i.e., presence of vitellogenic oocytes and stage-1 POCs).

Month	# active (non-spawners)	# of spawners	Proportion spawners
April	4	1	0.20
May	129	4	0.03
June	412	42	0.09
July	541	71	0.12
August	155	13	0.08
September	15	3	0.17
May - August	1237	130	0.01

Table 5.—Sex ratio by fork length for Gray Triggerfish collected off the southeastern U.S. Atlantic coast during 1994-1997 and 2009-2012. Chi-square analyses were performed in RStudio (RStudio 2013).

Fork length (mm)	Total number of fish	Male:Female	*P*
	1994-1997		
151-200	105	1:1.76	<0.05
201-250	327	1:1.42	<0.05
251-300	557	1:1.86	<0.001
301-350	737	1:1.21	<0.05
351-400	500	1:1.08	0.37
401-450	204	1:0.44	<0.001
451-500	48	1:0.07	<0.001
501-550	6		
551-600	1		
	2009-2012		
151-200	11	1:0.38	0.13
201-250	39	1:2	0.04
251-300	236	1:2.69	<0.001
301-350	417	1:2.16	<0.001
351-400	369	1:1.17	0.13
401-450	180	1:0.36	<0.001
451-500	39	1:0.18	<0.001
501-550	4	1:0.33	

Table 6.—Sex ratio by age for Gray Triggerfish collected off the southeastern U.S. Atlantic coast during 2009-2012. Chi-square analyses were performed in RStudio (RStudio 2013).

Age (years)	Total number of fish	Male:Female	*P*
0	2	1:1	
1	82	1:1.41	0.12
2	257	1:1.73	<0.001
3	327	1:1.32	<0.05
4	248	1:1.41	<0.05
5	157	1:1.09	0.58
6	67	1:1.39	0.18
7	30	1:0.88	0.72
8	18	1:2.6	0.06
9	7	1:2.5	
10	2	1:1	

APPENDIX

Appendix Table 1. Summary of statistics used during each time period.

Time Period	Null hypothesis tested	Analysis and program
1991-2012	No significant difference in mean size between males and females	Student's t-test; SPSS
	No significant difference in size frequency distribution between males and females	Kolmogorov-Smirnov (K-S test); SPSS
1994-1997 and 2009-2012	No significant difference in mean size between sexes and between time periods	Two-factor Analysis of Variance; SPSS
	No significant difference in size frequency distributions for females between the two time periods	K-S test; SPSS
	No significant difference in size frequency distributions for males between the two time periods	K-S test; SPSS
2004-2012	No relationship exists for males between size and depth of capture in NC-Area	Linear regression; SPSS
	No relationship exists for females between size and depth of capture in NC-Area	Linear regression; SPSS
	No relationship exists for males between size and depth of capture in SC-Area	Linear regression; SPSS
	No relationship exists for females between size and depth of capture in SC-Area	Linear regression; SPSS
	No significant difference in size frequency distributions for males between two depth zones of < 36 m and 40-65 m in NC-Area	K-S test; SPSS
	No significant difference in size frequency distributions for females between two depth zones of < 36 m and 40-65 m in NC-Area	K-S test; SPSS
	No significant difference in size frequency distributions for males between two depth zones of < 36 m and 40-65 m in SC-Area	K-S test; SPSS
	No significant difference in size frequency distributions for females between two depth zones of < 36 m and 40-65 m in SC-Area	K-S test; SPSS
2009-2012	No significant difference in mean age between males and females	Student's t-test; SPSS
	No significant difference in age frequency distribution between males and females	K-S test; SPSS
	No significant difference in the residuals of the von Bertalanffy growth parameters between males and females	Variance ratio test; RStudio

Appendix Table 2. Two-factor ANOVA for log-transformed mean sizes. Time period (2 levels: 1994-1997 and 2009-2012) and sex (2 levels: male and female) served as fixed factors in the model. Test was performed in SPSS (IBM Corp. 2012).

Source of variation	SS	df	MS	*F*	*P*
Period (P)	5.095	1	5.095	109.8	<0.001
Sex (S)	4.442	1	4.442	95.7	<0.001
P × S	0.032	1	0.032	0.7	0.403
Error	74.467	1605	0.046		
Total	53553.75	1609			