AGE, GROWTH AND REPRODUCTIVE BIOLOGY OF'THE GRAY TRIGGERFISH (Balistes capriscus) FROM THE SOUTHEASTERN UNITED States, 1992-1997

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COLLEGE OF CHARLESTON

AGE, GROWTH AND REPRODUCTIVE BIOLOGY OF THE GRAY TRIGGERFISH (*Balistes capriscus*) FROM THE SOUTHEASTERN UNITED STATES, 1992-1997

by

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ABSTRACT

Ages of 2,267 gray triggerfish from fishery-independent and fishery-dependent samples were estimated from sections of the first dorsal spine. Marginal increment analysis indicated that annual increment formation occurred in June for all age classes. Gray triggerfish ranged in age from 0-10 years, and in fork length (FL) from 82-560 mm. A total of 2,174 gonads was examined histologically and assigned a reproductive state. Males were significantly larger than females, and fish from fishery-dependent samples were significantly larger than fishery-independent specimens. Mature females from fishery-independent samples were found in 0% of age-0, 98 % of age-1 and age-2 fish, and 100% of fish older than age-3. Mature males from fishery-independent samples were present in 63% of age-1, 91% of age-2, 98% of age-3, 99% of age-4 and age-5, and 100% of older age fish. Females reached first maturity at 142 mm FL, with an L_{50} of 158 mm FL. Males first matured at 170 mm FL, with a L_{50} of 180 mm FL. No immature fish were sampled with fishery-dependent gear. The sex ratio was not significantly different from 1:1 for fishery-dependent samples. The sex ratio for fishery-independent samples was 1.2:1 in favor of females. Gray triggerfish are group-synchronous gonochorists. Female spawning frequency was identified by the presence of postovulatory follicles. Gray triggerfish spawn every 37 days, or 3-4 times per season. Female gray triggerfish were in spawning condition from April-August, with a peak of activity during June-July. Male gray triggerfish were found in spawning condition throughout the year; however, there was a peak in activity during May-September.

INTRODUCTION

The rocky outcrop and live bottom habitats of the continental shelf along the Atlantic coast of the southeastern United States support a wide variety of commercially and recreationally important fish species. These reef fish assemblages consist of subtropical and tropical demersal species of snappers (Lutjanidae), sea basses and groupers (Serranidae), porgies (Sparidae), tilefishes (Malacanthidae), grunts (Haemulidae), jacks (Carangidae), and triggerfishes (Balistidae) (Parker *et al.*, 1979; Grimes *et al.*, 1982; Sedberry and Van Dolah, 1984), which are managed collectively as part of the snapper-grouper complex by the South Atlantic Fishery Management Council (SAFMC).

The South Atlantic Fishery Management Council has used trends in fishery-dependent landings to assess the population status of various species important to the fishing industry (SAFMC, 1983). Amendment 6 of the snapper-grouper Fishery Management Plan was " . . . developed to protect and rebuild the hogfish, cubera snapper, and gray triggerfish resources" (SAFMC, 1994, p.1). A snapper-grouper stock or stock complex is considered to be overfished when it is below the level of 40% of the spawning stock biomass per recruit (SSBR) that would occur in the absence of fishing (SAFMC, 1997). Using this standard, SAFMC determined the gray triggerfish was overfished, with an SSBR of 27% in 1992 (SAFMC, 1994).

From 1978 to the present, the Marine Resources Monitoring, Assessment and Prediction (MARMAP) Program has collected fishery-independent data to assess the

status of reef fish stocks in the South Atlantic Bight (SAB) (McGovern and Machowski, 2000). Data were collected for several economically important species such as vermilion snapper (*Rhomboplites aurorubens*), red porgy (*Pagrus pagrus*), black sea bass (*Centropristis striata*), white grunt (*Haemulon plumieri*) and gray triggerfish. Trends in abundance of each species was determined using catch per unit effort (CPUE). Between 1983-1996, McGovern and Machowski (2000) reported a decrease in the relative abundance of vermilion snapper, black sea bass, and red porgy, and an increase in the abundance of white grunt and gray triggerfish. Gray triggerfish CPUE increased from 0.1 fish per trap hour in 1988 to 2.4 fish per trap hour in 1996 (McGovern and Machowski, 2000). However, fishery-independent CPUE for gray triggerfish has decreased since 1996 (McGovern and Machowski, 2000), which may be an early sign of increased fishing pressure that began in 1997. The decrease in fishery-independent CPUE appears to support the assessment by the 1992 SAFMC that gray triggerfish are overfished.

Very few studies on the age and growth of gray triggerfish have been published (Johnson and Saloman, 1984; Ofori-Danson, 1989), none of which was conducted off the Atlantic coast of the southeastern United States. Previous studies of reproductive biology in gray triggerfish (Aiken, 1975; Fricke, 1980; Ofori-Danson, 1990) utilized only macroscopic observation of female reproductive structures. Histological techniques are needed to assess sexual maturity and spawning activity. A description of the male reproductive system is needed as previous reproductive biology studies disregarded the male reproductive structures due to their relatively small size (Ofori-Danson, 1990).

This research was completed to help establish the current status of the gray triggerfish population from the Atlantic coast of the southeast United States. The objectives of the present study were to determine the ages, growth rates, reproductive morphology, sex ratio, size and age at first maturity, and spawning season of the gray triggerfish.

MATERIALS AND METHODS

Gray triggerfish were collected with fishery-independent sampling conducted from May through September of 1992 to 1997 throughout the SAB. Gray triggerfish were sampled using chevron traps at randomly-chosen reef sites in depths ranging from 20-110 m. Depth, latitude, longitude, sampling duration and time of day were recorded for each trap set (see Harris and McGovern, 1997 for sampling details). During 1992-1993, up to 15 gray triggerfish from each 1-cm size class, as well as all gray triggerfish sampled during 1994-1997, were retained for life history analysis. To supplement fishery-independent collections, 50-100 whole, unsorted gray triggerfish caught with commercial hook and line gear were purchased each month from local fish houses in North Carolina and South Carolina from October 1996 through October 1997.

For fishery-dependent and fishery-independent collections, each gray triggerfish was weighed to the nearest gram. Total length (TL), fork length (FL) and standard length (SL) were measured to the nearest mm with an electronic fish measuring board. The first dorsal spine was removed, stored in a coin envelope, and allowed to air dry for at least 7 d. The entire male gonad and a posterior section of the female gonad were retained for histological examination. Each gonad from fishery-dependent samples was weighed to the nearest 0.1 g.

Age and Growth

Sagittal otoliths were removed from five gray triggerfish in an attempt to verify annuli formation. The otoliths were extremely small (<2 mm), and were considered unreadable; therefore dorsal spines were used for age determination. Spines were sectioned using a Buehler® Isomet low-speed saw at 300 rpm. Two sections, immediately distal to the condyle groove (Fig. 1), were cut from each spine. Sections were mounted on glass slides with clear mounting medium and viewed using a Nikon® SMZ-2T dissecting microscope at 10-25X with transmitted light. The dissecting microscope was linked by a Hitachi® KP-550 video camera to a personal computer equipped with a MATROX® frame grabber and OPTIMAS® image analysis software (Optimas Corp., 1996).

Increments on spine sections were counted as one opaque zone and one translucent zone. Two readers independently counted spine increments on a section, without knowledge of fish length or date of capture. If counts differed between readers, the spine was examined by both individuals simultaneously and discarded if no agreement on age could be reached. The spine radius was defined as the distance between the focus of the spine section and the distal edge of the spine (Fig. 2). Radii were measured for both sides of 100 spines. No difference between sides was found (paired t-test; df=99, $p>0.19$); therefore, either side was considered suitable for measurement.

Each translucent zone was measured from the focus to the most distal edge of the increment. Marginal increment was defined as the difference between the radius of the spine and the measurement of the last increment used in age determination (Fig. 2). To

verify identification of the first increment, the distance from the focus to the edge of the first increment was measured for all age classes. Increment measurements were plotted for ages 1,3,5 and fishery-independent samples for both sexes to ensure that measurements of the first annulus were consistent across age classes.

For each age class, mean monthly marginal increment analysis was used to determine the periodicity of increment formation. Specimens from fishery-independent and fishery-dependent samples were pooled, and mean marginal increment was plotted by month. If the plot was unimodal, increment formation was considered to be annual in nature.

Fork length (FL) was used for all analyses since length of the tail filaments could be inconsistent. Analysis of variance (ANOVA) was used to compare FL between sexes and gear types (traps vs. commercial hook and line). The relationship between FL and total body weight (TBW) was also described.

Least squares linear regressions were used to determine the relationship between spine radius and FL for each sex and gear type:

$$L = c + b\,(SR),$$

where L = fork length

c = y-intercept

b = slope

SR = spine radius

Analysis of covariance (ANCOVA) was used to compare slopes and y-intercepts to determine if the separate regressions were needed for back-calculation of length at age.

Back-calculated lengths at age were calculated using the Fraser-Lee equation (Francis, 1990):

$$L_i = c + (L_c - c)\,(SR_i / SR_c),$$

where L_i = fish length at time of formation of the i^{th} increment

c = L-intercept from the equation $L = c + b(SR)$, the regression of FL on spine radius

L_c = fish length at time of capture

SR_i = spine radius at time of formation of the i^{th} increment

SR_c = spine radius at time of capture

The SigmaPlot curve-fitting module, with the Marquart-Levenburg algorithm (Jandel, 1995), was used to fit von Bertalanffy growth curves (von Bertalanffy, 1938) to unweighted mean observed FL and unweighted mean back-calculated FL for male and female gray triggerfish from fishery-dependent samples. Growth curves were also fitted to mean back-calculated lengths at age for males and females from fishery-dependent samples. Growth rates were also calculated from observed and back-calculated FL for both sexes and gear types.

Length at age was compared for fish caught during 1992 and 1997 from fishery-independent samples, to determine if all specimens could be combined to construct age-length keys. ANOVA was used to compare length at age for all age classes, sexes combined. Because there were no significant differences between years (ANOVA; df=1, $p<0.001$), data from 1992-1997 were pooled. An additional 300 specimens per year were randomly selected from fishery-independent samples during 1993-1996 to represent these sampling years. An age-length key was constructed for each gear type (all years

combined). Sexes were combined for age-length keys because male and female gray triggerfish are indistinguishable by external morphology.

Lengths at age for both sexes were examined for the effects of depth and latitude on fishery-independent specimens. Depth was divided into two zones (≤45 m and >45 m), using 45 m to represent the depth of the continental shelf break. Latitude was divided into three zones (<30°N, 30°-32°N, >32°N). Lengths at age were compared using a two-way ANOVA to examine the singular effect of each variable, as well as any possible interaction. Rejection of the null hypothesis was based on $\alpha = 0.05$ for all analyses.

Reproduction

Reproductive tissue from 2,147 gray triggerfish was examined microscopically and assigned a sex and stage of maturity. Whole gonads were removed from each fish from fishery-dependent samples. Gonad weight (±0.1 g) was taken for females, and a gonadosomatic index (GSI) was described with the formula:

$$GSI = \frac{\text{gonad weight (g)}}{\text{body weight (g)} - \text{gonad weight (g)}}$$

A monthly gonadosomatic index was calculated for both gear types combined to describe spawning activity.

The entire male gonad, as well as associated ducts and accessory structures, was placed in a Tissue-Tek® embedding capsule, and fixed in 11% seawater formalin buffered with marble chips. Whenever possible, the entire ovary was similarly fixed. If the ovary was too large, only the posterior section, containing portions of both lobes was placed in the embedding capsule. Occasionally, an ovary was so large that a transverse section of a single lobe was taken for histological analysis. After at least 14 d, tissue was transferred

to 50% isopropanol for a minimum of 14 d. The tissue samples were processed in a Modular Vacuum Processor, vacuum infiltrated and blocked in paraffin.

After processing, tissue samples were sectioned on a rotary microtome (7μm) and three sections of each gonad sample were mounted on a slide, which was stained with double-strength Gill hematoxylin and counter-stained with eosin-y. Stained sections were viewed under a compound microscope at 40-400X to determine sex and reproductive state. The author served as first reader on fishery-dependent samples, and assigned sex and reproductive state to all sections, as well as 30% of fishery-independent samples. A second reader independently assigned sex and reproductive states to all fishery-independent samples and 30% of the fishery-dependent samples. If differences in maturity assignments occurred, both readers reread the slide simultaneously. If no decision could be reached, that specimen was eliminated from analyses.

To examine male reproductive morphology, whole, fixed gonads from male gray triggerfish were preserved in 50% isopropanol. Instead of three cross-sections, some specimens were sectioned lengthwise to examine the structure of testes, as well as associated ducts. Lengthwise sections were stained and mounted using the same techniques previously described.

Samples were classified according to a modified version of the histological criteria used by Harris and McGovern (1997) (Table 1). To ensure that immature and resting specimens were assigned correctly, the frequencies of immature, definitely mature (developing, spawning and spent) and resting fish for each length class were compared. If there was little or no overlap in FL of immature and resting specimens, it was assumed that the stages were assessed correctly.

Spawning activity in females was identified by the presence of postovulatory follicles (POFs), and stages were assigned according to the level of degeneration present in the follicle. Stage 1 POFs showed very little degeneration, large size, distinct thecal and granulosa layers, and a highly convoluted lumen (Fig. 3A). Stage 2 POFs were smaller, showed degeneration of the granulosa and thecal layers, and a less distinct lumen (Fig. 3B). Stage 3 POFs showed a characteristic triangular shape of the granulosa layer, an often indistinct thecal layer and a reduced or absent lumen (Fig. 3C).

To calculate spawning frequency, the methods of Fitzhugh *et al.* (1993) and Cuellar *et al.* (1996) were used to calculate overall percentages of stage-2 and stage-3 POFs. Stage-1 POFs were eliminated from analysis, due to sufficient sample size. A proportion was computed for each category by dividing the total number in each category by the total number of females with vitellogenic oocytes. An average proportion was then calculated, and multiplied by the number of days in the spawning season. Spawning season was defined as the date (10 Apr 1997) when POFs first appeared in a specimen until the latest date (22 Aug 1997) when POFs appeared in a specimen.

A chi-square test was used to determine if sex ratios for each gear type, size class and age class were significantly different from 1:1. The percentage of mature individuals by size and age intervals was calculated for each sex. To estimate length at 50% maturity (L_{50}), SAS PROBIT analysis (SAS Institute, Inc., 1990) was used for maturity data in 25-mm intervals. The LOGISTIC procedure was used to determine which model (probit, logit, normit) to fit to the data. Logit and probit models were fit to data from females and males, respectively. Because no immature fish were captured with fishery-dependent gear, L_{50} analysis was performed only on fishery-independent data.

RESULTS

Age and Growth

During 1992 through 1997, 2,510 gray triggerfish were sampled for life history analysis. Of these fish, 1,743 were from fishery-independent samples (Fig. 4) and 767 were from fishery-dependent samples. A total of 2,263 fish were aged (1,533 from fishery-independent samples and 730 from fishery-dependent samples) and 9.8% of gray triggerfish dorsal spines were unreadable. Initial agreement between readers (± 1 increment) was 94%. Marginal increment analysis indicated that increment formation was annual, with increment formation occurring in June for all age classes (Fig. 5).

Females captured with fishery-independent gear ranged in age from 0-9 years and 82-560 mm FL, while males ranged in age from 1-9 years and 136-534 mm FL (Fig. 6). Females from fishery-dependent samples ranged from 3-9 years and 254-465 mm FL, while males ranged from 3-10 years and 277-511 mm FL (Fig. 6). There was a strong relationship between FL and TL (Table 2), FL and SL (Table 3) and FL and TBW (Table 4) for both sexes, regardless of gear type.

Fish sampled with traps had significantly smaller fork lengths than fishery-dependently caught fish (ANOVA; $p<0.001$, $df=1$), regardless of sex. Male gray triggerfish were significantly larger than female gray triggerfish for both gear types (ANOVA; $p<0.001$, $df=1$). Due to these differences in size as a result of sex and gear type, all data analyses, except age-length keys, were performed separately by sex and gear type.

The linear regressions of FL on SR resulted in similar r^2 values between fishery-independent and fishery-dependent samples for both sexes (Fig. 7).

Males (fishery-independent): $FL = (0.01)SR + 1.13$, $n = 674$, $r^2=0.33$

Males (Fishery-dependent): $FL = (0.01)SR + 1.64$, $n = 330$, $r^2=0.32$

Females (fishery-independent): $FL = (0.01)SR + 0.80$, $n = 805$, $r^2=0.35$

Females (fishery-dependent): $FL = (0.01)SR + 2.29$, $n = 354$, $r^2=0.24$

Slopes for the FL/SR relationship were significantly different for gray triggerfish taken from fishery-independent or fishery-dependent samples, regardless of sex (ANCOVA, $p<0.001$, $F=337.25$, $df=3$); therefore, separate regressions were used to define all FL-SR relationships. Since measurements of the first annulus were consistent for all age classes, it was determined that the first annulus was being assigned correctly (Figs. 8, 9).

A comparison of observed lengths at age indicated that fishery-dependent specimens were larger at age than fishery-independent specimens at ages 3 and 4, regardless of sex (Figs. 10, 11; Tables 5, 6). Back-calculated age-1, age-2 and age-3 fish from fishery-dependent samples were significantly larger than those from fishery-independent samples for both sexes (ANOVA, $p<0.001$, $df=1$). Furthermore, male gray triggerfish reached larger sizes at age than female gray triggerfish, regardless of gear type (ANOVA, $p<0.001$, $df=1$).

No fish younger than age 3 were sampled with fishery-dependent fishing gear; therefore, the von Bertalanffy growth curve was fitted to unweighted mean back-calculated lengths at age, and failed to reach an asymptotic growth for the ages sampled, regardless of sex or gear type for the ages sampled (Figs. 12, 13). However, values for

L_∞ derived from the von Bertalanffy equations were larger for males than for females, regardless of gear type (Tables 7, 8).

Growth rates calculated from observed length at age from gray triggerfish from fishery-independent and fishery-dependent sampling declined with increasing age (Figs. 14, 15). Triggerfish exhibited rapid growth during the first year of life, after which growth began to slow down. Observed and back-calculated lengths at age show that male gray triggerfish grow more rapidly than females during the first two years, regardless of gear type (ANOVA, $p<0.001$, $df=1$).

Length at age varied significantly with depth of capture for male and female gray triggerfish sampled with fishery-independent gear (Table 9). Larger gray triggerfish were found at depths greater than 45 m, and smaller fish were captured in shallower depths for ages 3, 4, 5 and 7 for males and ages 3-7 for females (Table 9). Latitude comparisons showed no effect of latitude on fork length (two-way ANOVA, $p=0.36$, $df=2$).

Separate age-length keys were constructed for specimens caught with fishery-independent and fishery-dependent gear, with sexes combined (Tables 10, 11). These keys were divided into 1-cm size classes, and showed a wide range of ages within size classes. For example, in fishery-independent samples, ages of gray triggerfish that were 35 mm FL range from 2-9, ages of gray triggerfish that were 19 cm FL ranged from 0-5.

Reproduction

Fork length frequency histograms of immature, definitely mature and resting male and female gray triggerfish showed minimal overlap between immature and resting specimens, regardless of sex (Fig. 16). Therefore, it was determined that immature and

resting stages were assigned correctly. Gray triggerfish were identified as a gonochoristic species with group-synchronous oocyte development and no more than three to four batches of oocytes in the ovary at one time. These consisted of a population of larger, vitellogenic oocytes, and a population of oocytes in the cortical alveoli stage, from which the next clutch is recruited. Female gray triggerfish spawned every approximately 15 days, or 8-9 times per spawning season (Number of specimens by stage: 30 stage-2 POFs, 32 stage-3 POFs, 484 total vitellogenic).

The overall sex ratio for fish from fishery-independent samples was significantly different from a 1:1 ratio in favor of females (Table 12). Females were significantly more predominant in 251-275, 301-325 and 326-350 mm FL size classes. Males significantly outnumbered females in the all size classes larger than 400 mm FL. The overall sex ratio of specimens caught with commercial gear did not significantly differ from a 1:1 ratio (Table 13). However, similar to fish caught with fishery-independent gear, females significantly outnumbered males in all size classes less than 325 mm FL and males significantly outnumbered females in size classes greater than 400 mm FL. There were no significant differences from a 1:1 sex ratio within age classes, regardless of gear type ($\chi 2$, $p>0.05$, $df=1$).

Sexually mature females from fishery-independent samples were found in 0% of age-0, 98% of age-1, 100% of females age-2 and older (Table 14). Mature males from fishery-independent samples were present in 63% of age-1, 91% of age-2, 98% of age-3, 99% of age-4 and age-5 and 100% of fish age 6 and older (Table 15). The smallest mature female was 142 mm FL, with a L_{50} of 158 mm FL (Logit model, 95% CI = 111-169 mm FL). Males first reached maturity at 170 mm FL, with a L_{50} of 180 mm FL

(Probit model, 95% CI = 170-187 mm FL). Immature females ranged from 82-185 mm FL and immature males ranged 136-228 mm FL. No immature fish were sampled from fishery-dependent catches.

No females with hydrated oocytes (running ripe) were present in the gray triggerfish sampled. Females were in spawning condition from April to August, with the greatest amount of activity during June-July (Fig. 17). GSI values were greatest in June (Fig. 18), which indicated a period of peak spawning. Male gray triggerfish were in spawning condition throughout the year, with peak activity during June-September (Fig. 17).

Male Reproductive System

The testes of the male gray triggerfish are separate, oval-shaped structures that lay close together along the ventral side of the swim bladder (Fig. 20A). Unlike most teleost fishes, the testes of the gray triggerfish do not join at the posterior end, but remain separate throughout their length. Spermatogenesis is atypical, in that a majority of lobules are filled with spermatocytes, while a small number of spermatocysts contain advanced spermatozoa (Fig. 21). The lobules of the testes are drained via efferent ducts into the main testicular duct, which in positioned along length of each testis (Fig. 20B). Some efferent ducts of each testis extend externally beyond the posterior end of each testis, at which point the efferent ducts and testicular ducts of both testes fuse to become the common spermatic duct (Fig. 22B). Unlike most teleosts, the common spermatic duct in gray triggerfish is lined with secretory epithelial cells.

The common spermatic duct is surrounded by a bilateral outgrowth with tubules and secretory ducts, a structure that has been called an accessory gland in gobies and blennies. The weak staining with hematoxylin and eosin-y of the tissue that surrounded these ducts suggests a secretory function. This accessory gland is a unique structure in that the structure and function resembled both a seminal vesicle and an epididymus of higher vertebrates. The accessory gland is connected to the ventro-posterior surface of the urinary bladder duct via connective mesenteries along the outgrowth (Fig. 22A). In male gray triggerfish, the urinary bladder is an elongated structure that lay in close proximity to the accessory gland throughout its length (Fig. 22A).

The secretory function of the accessory gland is carried out by secretory epithelium, which lines all ducts and varies in form, depending on stage of reproductive cycle. While the male is in the resting stage, the cells of the epithelium are cuboidal and relatively inactive. As spermatogenesis continues toward spawning, the cells become columnar, which indicates an increase in secretory function.

The atypical morphology and physiology of the male reproductive system of gray triggerfish resulted in the addition of two new reproductive stages, storage and recent spawn (Table 1). The storage stage is present in individuals that have completed development and are storing spermatozoa in the ducts of the accessory gland, which are rounded and densely packed with spermatozoa (Fig. 19A). Orientation of the spermatozoa, with tails normal to the duct walls, indicates that spermatozoa are being stored, not released (Fig. 21). The epithelial cells of the accessory gland appear to be secreting fluids to nourish the spermatozoa during the storage stage.

The running ripe stage, typical of marine teleosts, when testes and ducts are filled with spermatozoa, was not observed in any of the males sampled. The recent spawn stage was present in 21% of the males examined and was used to identify spawning activity. During this stage, the ducts of the accessory gland are extremely enlarged and partially filled with residual spermatozoa (Fig. 19B). Upon completion of spawning, epithelial cells lining the ducts appear columnar and increase production of phagocytic granules, which are secreted into the lumen of the accessory gland ducts. These phagocytic cells serve to break down and reabsorb materials from the remaining spermatozoa. Phagocytosis continues through the spent stage, which is identified by the shrunken appearance of the ducts (Fig. 19C). During the resting stage, the epithelial cells return to their inactive, cuboidal shape and the ducts of the efferent system are small and rounded.

DISCUSSION

Age and Growth

Dorsal spines of gray triggerfish proved to be suitable for use in age determination. This was supported by the verification of annular increment formation by marginal increment analysis. Gray triggerfish are relatively short-lived, not exceeding 10 years of age. This reduced complications often associated with ageing older fish, including variable increment formation and increment crowding (Hubert et al., 1987; Pikitch and Demory, 1988; Braaten et al., 1999). There was also a significant relationship between spine radius and fork length; therefore, the first dorsal spine was considered the most reliable structure for age determination.

Gray triggerfish sampled with fishery-independent gear displayed a much wider range of lengths than those captured with fishery-dependent gear. None of the fish sampled with fishery-dependent gear was smaller than 254 mm FL. Similarly, gray triggerfish sampled commercially in the Gulf of Mexico ranged from 209 to 640 mm (Hood and Johnson, *in press*). Gray triggerfish have relatively small mouths, unlike groupers and sea basses, and therefore would not be vulnerable to the hooks of the fishery-dependent gear until they reached a relatively large size.

Gray triggerfish are relatively fast-growing fish, with the greatest growth rate during the first year of life. Male gray triggerfish from fishery-independent samples reach observed lengths of 217 mm FL and females reach 219 mm FL during the first year. Hood and Johnson (*in press*) reported a mean observed length of 224 mm FL for

female gray triggerfish and 268 mm FL for male gray triggerfish. Similarly, Johnson and Saloman (1984) found that male and female gray triggerfish in the Gulf of Mexico reach a mean observed length of 260 mm FL during the first year. The smaller length at age for triggerfish from the SAB may the result of sampling gear. Age-1 fish from the SAB were only found in fishery-independent samples, whereas all sampling from the Gulf of Mexico (Johnson and Saloman, 1984; Hood and Johnson, *in press*) were sampled from fishery-dependent sources. The commercial fishery may be selectively removing faster-growing fish from the population, resulting in larger length at age for those specimens.

The size selective nature of the commercial industry was evident in the comparison of back-calculated and observed lengths at age between gear types. Back-calculated lengths at age were significantly larger for fish from fishery-dependent samples than for fish from fishery-independent samples at ages 1-3. The larger back-calculated lengths at age indicated that the commercial fishery was targeting fish that are predisposed to rapid growth. Continued removal of faster growing individuals by commercial gear could result in smaller size at age (Plan Development Team, 1990; Harris and McGovern, 1997).

The size-selective nature of the fishery-dependent data was also evident in the age structure of the fishery-dependent samples. No fish younger than age three was captured with commercial fishing gear. Similarly, Hood and Johnson (*in press*) found that gray triggerfish in the Gulf of Mexico were fully recruited to the commercial fishery by age 4. The range of lengths sampled with fishery-independent gear indicates that this trapping gear is less size-selective, capturing both slow-growing and fast-growing gray triggerfish; thus, providing a better estimate of growth in the entire population.

The growth curves derived from mean back-calculated lengths at age allowed for comparisons between growth trends in each gear type. Von Bertalanffy growth curves could not be fitted to observed length at age for males and females from fishery-dependent samples due to the fact that no fish were sampled under the age of three.

Male gray triggerfish attained larger size at age than females, regardless of gear type, which may be crucial for reproductive success. The territorial and competitive behavior of male and female triggerfish, reported by Fricke (1980), Ishihara and Kuwamura (1996), and Gladstone (1994), plays a critical role in gray triggerfish reproduction. Male gray triggerfish have been observed with harems consisting of as many as three females, and are responsible for defending the nests of the females from fertilization by other males (Ishihara and Kuwamura, 1996). A larger size would make male gray triggerfish more competitive insuring increased reproductive success, as larger fish are more likely to provide a better defense.

The von Bertalanffy growth curves obtained from back-calculated lengths further demonstrated that male gray triggerfish obtained larger mean length than females. The theoretical maximum lengths that resulted for males from fishery-independent and fishery-dependent (486 mm FL and 489 mm FL, respectively) were larger than those calculated for females (412 mm FL and 430 mm FL). Estimates of L_{∞} for gray triggerfish from fishery-dependent samples were similar to values reported by Johnson and Saloman (1984), which were 438 mm FL for females and 492 mm FL for males.

Gray triggerfish sampled in deeper waters attained larger length at age than those sampled in shallower waters, regardless of sex. This size-depth trend has been reported for both freshwater (Helfman, 1978; Power, 1984) and marine (Macpherson and Duarte,

1991) fishes. In the marine ecosystem, this larger-deeper phenomenon appears to be the result of offshore ontogenetic migration (Macpherson and Duarte, 1991). Several species of triggerfish are reported to be territorial (Fricke, 1980; Gladstone, 1994; Ishihara and Kuwamura, 1996; Kuwamura, 1997), but larger fish may establish territories in deeper waters than smaller fish.

Gray triggerfish are pelagic during the first year of life (Dooley, 1972; Aiken, 1975), and then settle to the bottom, inhabiting areas of live bottom and rocky outcrop substrates. Similar behavior was reported in the queen triggerfish, *Balistes vetula*. Robertson (1988) indicated that settlement of juvenile queen triggerfish directly affected the subsequent adult populations in the same site, suggesting that once settled, most remain in approximately the same location throughout their lives.

Although some gray triggerfish may move to deeper waters with ontogeny, the larger-deeper phenomenon may be due to other physiological or ecological factors. Gladstone (1994) reported that yellowmargin triggerfish temporarily move to deeper waters for the purpose of establishing spawning territories, remaining at the spawning grounds for 1-3 d, after which it leaves the territory until the next spawning cycle. Gladstone (1994) also observed larger males chasing away smaller conspecific males from the territory. If similar behavior is present in the gray triggerfish, sampling over spawning grounds may result in catches of the larger gray triggerfish that have established temporary territories in that area.

Reproduction

Modification of the reproductive classification system was necessary for female and male gray triggerfish because previous classification systems for female fish have included a running ripe stage, identified by the presence of hydrated oocytes (see Wyanski, et al., 2000). This condition was not found in any female gray triggerfish sampled, and may be the result of several factors. In marine teleosts that spawn pelagic eggs, hydration of the oocytes makes them buoyant in seawater (Wallace and Selman, 1981). If gray triggerfish are demersal spawners, hydration would not be necessary, explaining the lack of running ripe females.

If gray triggerfish do hydrate oocytes, the lack of running ripe females may be the result of sampling protocol. All sampling gear used was dependent upon bait to attract fish. Immediately prior to spawning, the ovaries of the female gray triggerfish are extremely enlarged, potentially filling the body cavity. Females may not have the desire to feed during the hours prior to spawning, and would not be vulnerable to fishing gear. Similar behavior was reported by Fricke (1980), who found that females did not feed for the duration of spawning and broodcare.

The lack of running ripe females may also be the result of the time of sampling. All fishery-independent sampling (for which time of capture was recorded) was conducted during daylight hours. Gladstone (1994) reported that the yellowmargin triggerfish (*Pseudobalistes flavimarginatus*) spawned in the early morning hours, with the first appearance of eggs by 0630 h. Similarly, Kuwamura (1997) reported that the blackbar triggerfish (*Rhinecanthus aculeatus*) spawns just after sunrise. If gray

triggerfish spawn in the hours around dawn, no running ripe females would be captured in fishery-independent sampling.

Another possible explanation for the lack of running ripe ovaries may be the result of trapping procedures. Traps are retrieved at a rapid rate, which may result in some degree of anatomical damage, caused by a rapid change in pressure. Rogers et al. (1986) examined patterns of trauma resulting from trawling procedures. Two species of the Balistidae were examined, and both showed unusually high rates of intestinal protrusion from the cloacal area. This phenomenon was attributed to the restricted pharyngeal area and bony sternum of balistid morphology. Due to the enlarged size of the female gonad during spawning condition, portions of the ovary may be extruded in addition to the intestines, possibly resulting in the loss of oocytes. If a running ripe stage exists in the gray triggerfish, the gonad may not remain intact during the retrieval of fishing gear, as a result of its enlarged size.

The testes of male gray triggerfish do not exhibit a true running ripe stage, common in other teleost fishes. A running ripe male in other teleosts is characterized by a large amount of spermatozoa in enlarged lobules within the testes and in the spermatic duct (White *et al.*, 1998). Instead, spermatozoa are stored in an accessory gland that is similar in structure and function to both the seminal vesicle and epididymus of higher vertebrates. Once spermatozoa develop, they are released into the accessory gland for storage prior to spawning.

Structures of this type have been described in a variety of teleost families, such as the Blenniidae, Gobiidae, Claridae, Bagridae and Tripterygidae (Fishelson, 1991). This gland can take several forms, from a long, fingerlike extensions of the ducts to bilateral

tubular outgrowths of the common spermatic duct. The accessory gland of the gray triggerfish resembles the latter. This type of gland has been described in gobies (Fishelson, 1991) as "richer in tubule and crypts but form an integral part of the enlarged spermatozoa-duct," which very closely resembles the duct structure in the male gray triggerfish.

Secretory cells line the accessory gland, and may serve several purposes in the reproductive system of male gray triggerfish. Weisel (1949) concluded that the "secretory product of the accessory glands performs the function of immobilization of the spermatozoa, thus facilitating their prolonged storage in the sex tract." Immediately prior, and often following, spawning activity, the spermatozoa in gray triggerfish appeared more dilute, which was almost always evident by lighter staining of histological samples. Secretions of the epithelium during this stage may serve to "activate" spermatozoa from the inactive storage condition to a mobile state for spawning. Eggert (1931) assumed that the secretions of the accessory gland served to improve the mobility of spermatozoa. Further work needs to be performed to determine the exact function of the accessory gland and the secretory substances.

There is a close association between the accessory gland and the urinary bladder, which may indicate a function of the bladder in reproduction. Rasotto and Sadovy (1995) report that the mucins secreted by the epithelial cells of the male urogenital system are believed to play a specialized role in breeding activity. For example, they are thought to facilitate nest building by increasing the viscosity of seminal fluid for demersal spawning (Hickman and Trump, 1969; Lahnsteiner *et al.*, 1990, 1992). This could explain the role of the urinary bladder in the reproduction of gray triggerfish.

Gray triggerfish reproductive biology exhibits patterns and trends that are unusual among reef fish species (Thresher, 1984). Among triggerfishes, some species exhibit maternal care, some bi-parental and some have both (Fricke, 1980; Gladstone, 1984). Care can take many forms, including nest defense and removal of sand and rubble from the nests and eggs. Parental care typically lasts for 24-48 hours after fertilization. This type of parental input may result in fewer reproductive events than in species with broadcast spawning, and longer periods of time between spawning events. For example, the vermilion snapper, *Rhomboplites aurorubens*, spawns approximately once every 5 days, or about 35 times a year (Cuellar et al., 1996) compared to once every 17-18 days, or 7-8 times a year in the gray triggerfish.

Spawning activity in gray triggerfish in the SAB was greatest during the summer for both sexes and gear types, which is similar to results obtained by Ofori-Danson (1990) for gray triggerfish in the eastern Atlantic. Male gray triggerfish in storage condition were sampled throughout the year in the SAB. If a male did not have a nest to fertilize, it may have continued to store spermatozoa in anticipation of spawning with another male's mate. This behavior has been called sneak spawning, and has been reported in several species of demersal spawning teleosts (Magnhagen, 1998; Scaggiante *et al.*, 1999; Mazzoli *et al.*, 2000). Scaggiante (1999) reported that the main function of the seminal vesicle is storage in smaller fish, and these fish are more likely to exhibit sneak spawning behavior, which could explain the existence of the storage stage throughout the spawning season. Sneak spawning is probably a relatively infrequent occurrence, which is supported by the relatively low numbers of males captured in the storage stage during October-March.

The territorial and aggressive behavior exhibited by the male gray triggerfish during spawning activity may help to explain the larger size of males at first maturity. Males reached maturity at 170 mm FL, compared to 142 mm FL for females. In addition, 100% of females were mature by 185 mm FL, compared to 228 mm FL by males. Hood and Johnson (*in press*) found that females did not reach 100% maturity until 400 mm FL, which is much larger than estimates from this study. This may be the result of sampling, which was solely fishery-dependent in the Gulf of Mexico. Hood and Johnson (in press) sampled only 14 immature females, which may have resulted in an overestimation of size at maturity.

Larger size at maturity may be crucial for males, who are forced to establish and defend nests. Smaller mature males may be unable to establish territories or attract females; therefore, resorting to sneak spawning whenever possible. Gladstone (1994) reported incidences of smaller conspecific males being chased away from nests by the resident male, indicating the benefit of larger size in reproduction.

The difference in sex ratio noted between fishery-dependent and fishery-independent samples is the result of greater size-selectivity in commercial sampling gear. The smaller size classes were not well represented in fishery-dependent samples, and because females dominated in the smaller size classes, the absence of smaller fish in the commercial samples may have underestimated the number of females in the population. Results obtained by Hood and Johnson (*in press*) from fishery-dependent sampling showed a significant difference from a 1:1 ratio, in favor of males (3.01:1). Hood and Johnson (*in press*) also found that the ratio of males increased with increasing length.

SUMMARY AND CONCLUSIONS

Gray triggerfish sampled from the South Atlantic Bight (SAB) during 1992-1997 ranged in age from 0-10 years and in length from 82-560 mm FL. First dorsal spines were suitable for use in age determination and increment formation, which occurred in June, was annual in nature. Observed and back-calculated lengths at age for fish sampled with fishery-dependent gear were significantly larger than those for fish from fishery-independent samples. Males were significantly larger than females, regardless of gear type. It was determined that von Bertalanffy growth curves derived from back-calculated lengths gave a better depiction of growth in the population. Growth curves indicated that fishery-dependent specimens attained larger maximum length than fishery-independent specimens, regardless of sex, and that male gray triggerfish reached larger maximum length than females, regardless of gear type. Larger gray triggerfish were found at depths ≥45 m, regardless of sex.

Mature females were found in 0% of age-0, 98 % of age-1 and age-2, and 100% of fish age-3 and older. Mature males from fishery-independent samples were present in 63% of age-1, 91% of age-2, 98% of age-3, 99% of age-4 and age-5, and 100% of fish age-6 and older. Females were first mature at 142 mm FL, with a L_{50} of 158 mm FL. Males were first mature at 170 mm FL, with a L_{50} of 180 mm FL. No immature fish were sampled with fishery-dependent gear. The overall sex ratio was not significantly different from 1:1 for fish sampled with commercial gear; however, overall sex ratio for

fish from fishery-independent samples was significantly different from 1:1 in favor of females. For both gear types, females were predominant at smaller lengths, and males were predominant at larger lengths, indicative of larger size at age and larger maximum length of male gray triggerfish. There was no significant difference from a 1:1 sex ratio within age classes, regardless of gear type. If the fishery-dependent industry is selectively targeting the larger, faster-growing fish, it may also be selectively removing males from the population. Over time, this may skew the sex ratio in favor of females. The gonadosomatic index, as well as the presence of POFs, indicated a spawning season from April to August, with a peak during June-July. Male gray triggerfish appeared to be in spawning condition year round; however, there was a peak of activity in June-September.

The uniqueness of the gray triggerfish reproductive biology resulted in a modification of established reproductive criteria, as well as a detailed description of the male reproductive system. The addition of the storage and recent spawn stages helped identify spawning activity in males. The lack of running ripe males and females supported the idea that gray triggerfish are territorial by nature and exhibit demersal spawning behavior, which makes this species unusual among fishes in the snapper grouper complex. Decreasing fishery-independent CPUE, as well as the SSBR of 27% calculated by the SAFMC, may be early indications of fishing pressure in the gray triggerfish; therefore, management strategies should be enacted before fishing pressure reduces the population of gray triggerfish in the South Atlantic Bight.

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Figure 1. Lateral view of the first dorsal spine of the gray triggerfish, showing location of cuts for each section.

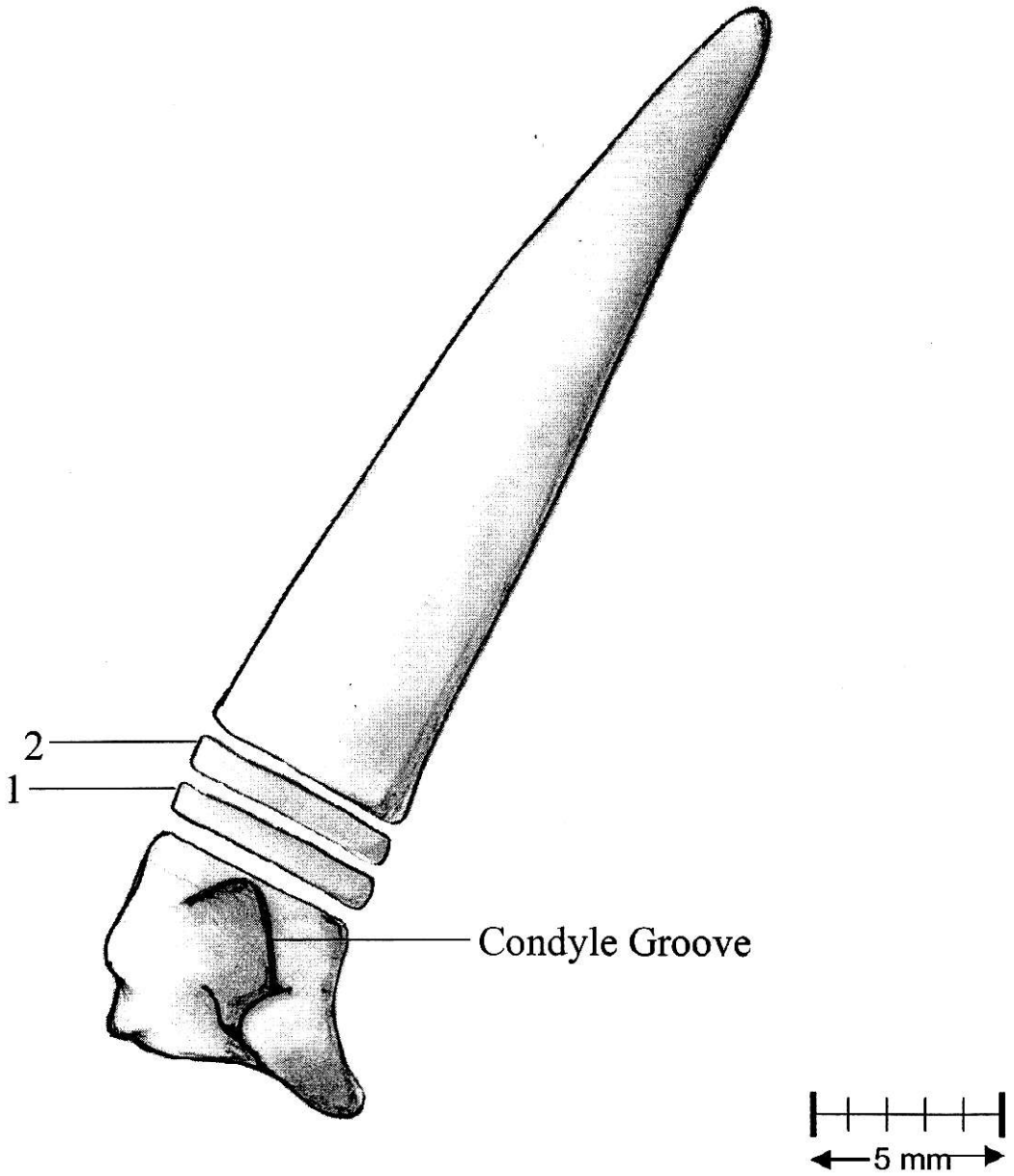

2
1
Condyle Groove
5 mm

Figure 2. Transverse section of a gray triggerfish dorsal spine depicting measurements of spine radius (SR), first annulus distance (A1), and marginal increment (MI).

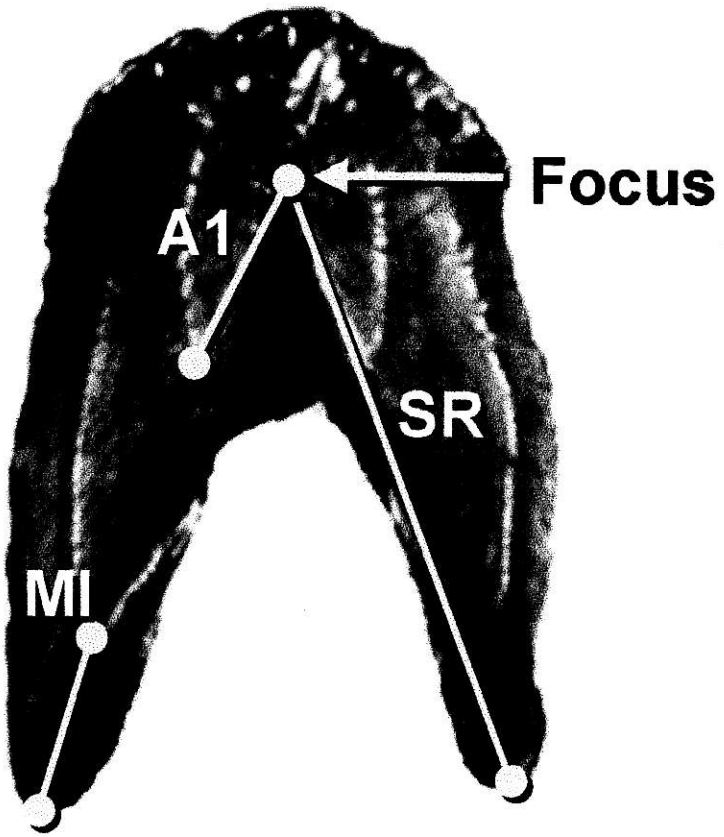
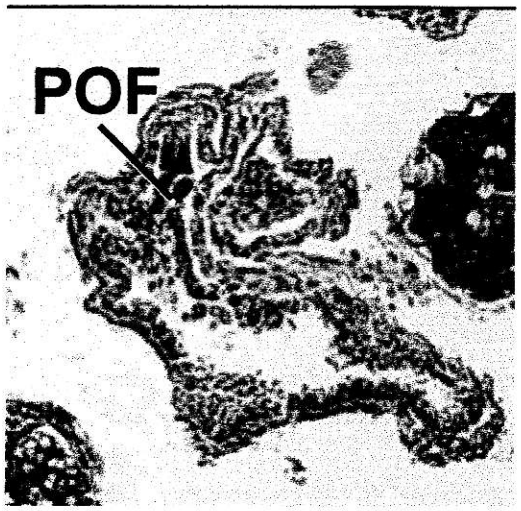
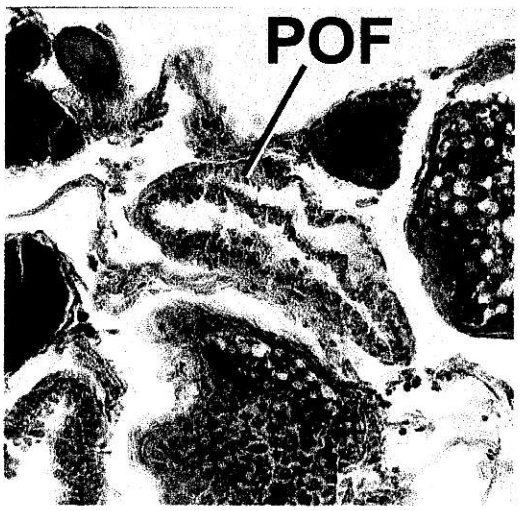

Focus
A1
SR
MI

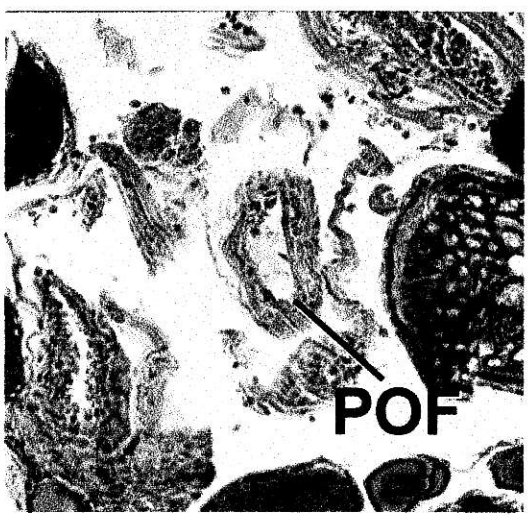
Figure 3. Photographs of stage 1 (A), stage 2 (B), and stage 3 (C) postovulatory follicles from female gray triggerfish.



(A)



(B)



(C)

Figure 4. Locations where gray triggerfish were captured with fishery-independent gear during 1992-1997. Isobaths represent 20m, 40m, 60m, 80m, 100m, 200m and 400m depths.

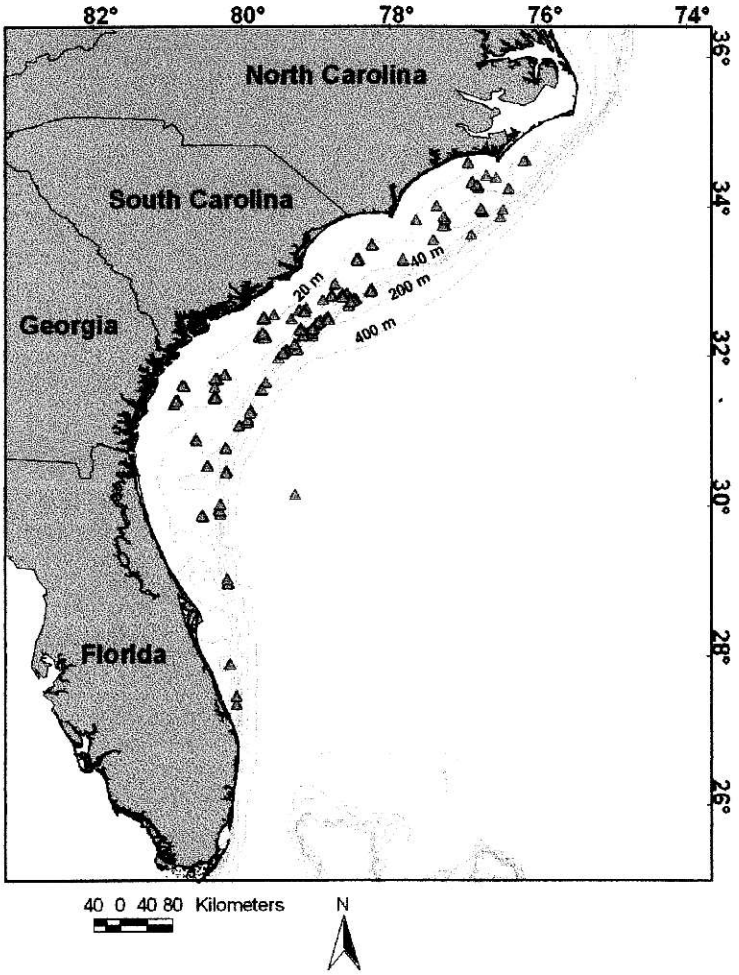

82°
80°
78°
76°
74°
36°
34°
32°
30°
28°
26°
North Carolina
South Carolina
Georgia
Florida
20 m
40 m
200 m
400 m
40 0 40 80 Kilometers
N

Figure 5. Mean marginal increment by month for ages 3-7 (all years combined) for gray triggerfish from fishery-independent and fishery-dependent samples.

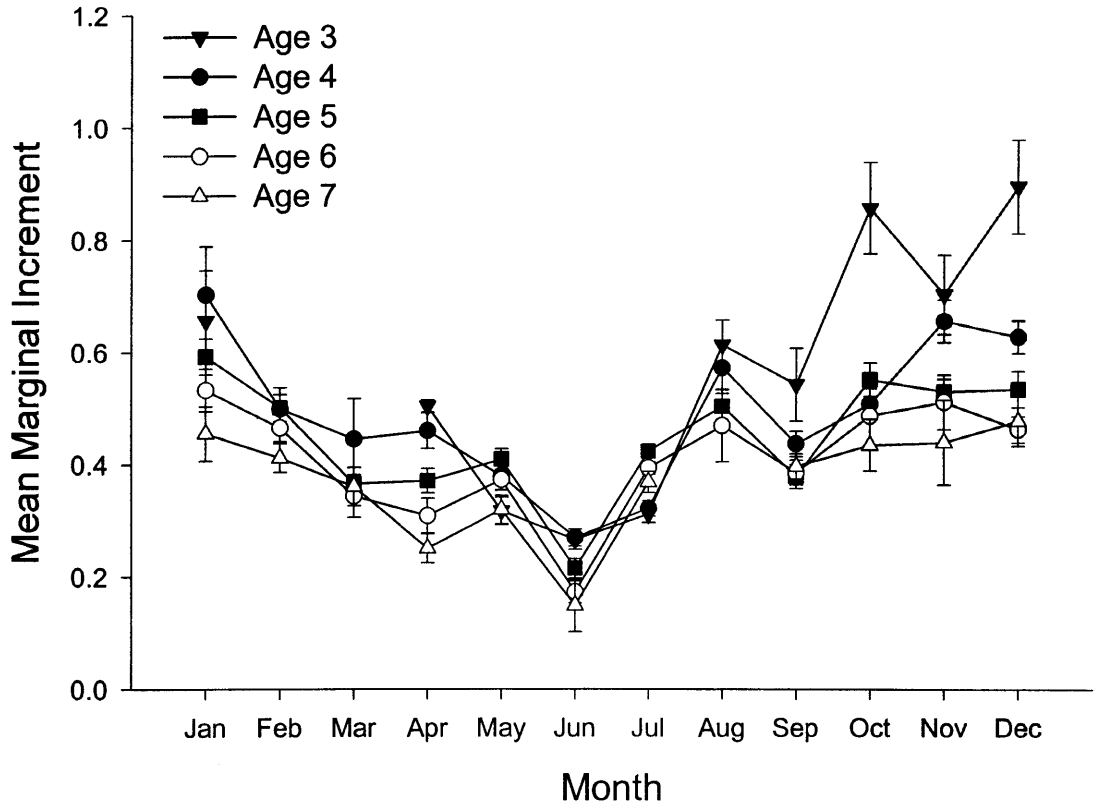

Age 3
Age 4
Age 5
Age 6
Age 7
Mean Marginal Increment
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month

Figure 6. Length-frequency distributions for males and females from fishery-independent samples during 1992-1997, and fishery-dependent samples during 1996-1997.

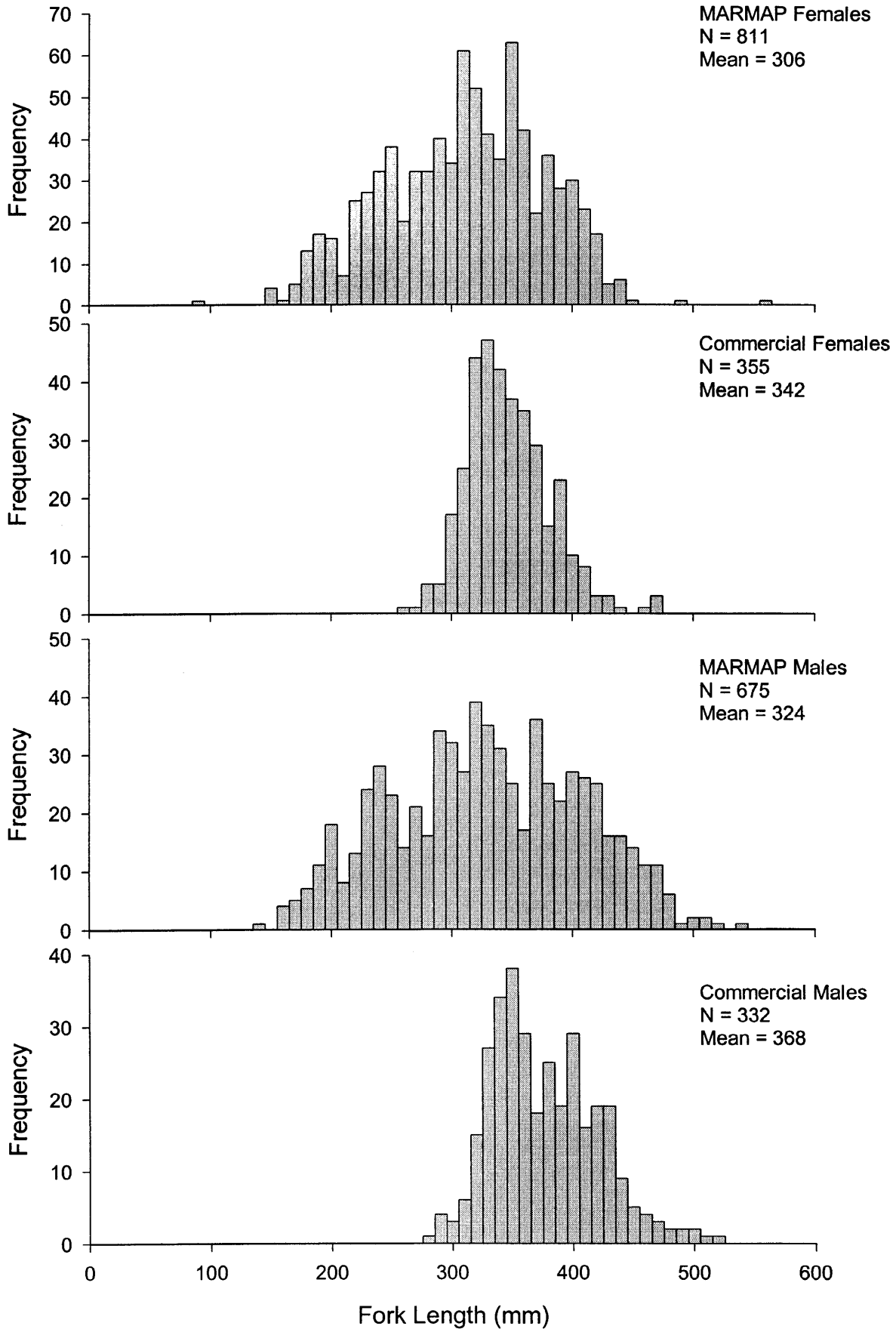

MARMAP Females
N = 811
Mean = 306
Commercial Females
N = 355
Mean = 342
MARMAP Males
N = 675
Mean = 324
Commercial Males
N = 332
Mean = 368
Frequency
Fork Length (mm)
0
100
200
300
400
500
600

Figure 7. Relationship of fork length and spine radius for males and females from fishery-independent samples from 1992-1997 and fishery-dependent samples from 1996-1997. Lines represent linear regressions.

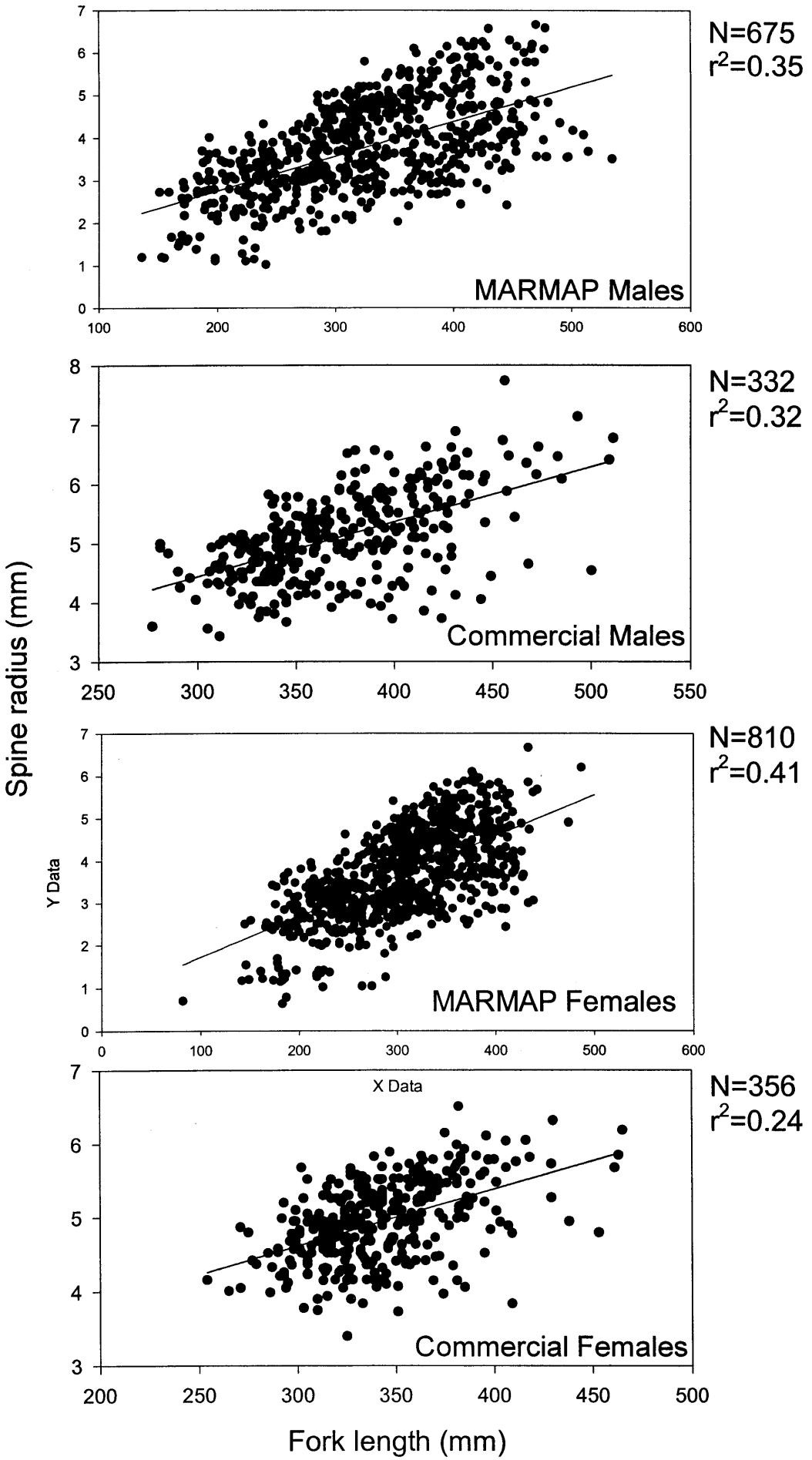

N=675
r2=0.35
MARMAP Males
N=332
r2=0.32
Commercial Males
N=810
r2=0.41
MARMAP Females
N=356
r2=0.24
Commercial Females
Spine radius (mm)
Fork length (mm)
Y Data
X Data

Figure 8. Frequency of increment measurements for male gray triggerfish ages 1,3,5 and 7 from fishery-independent samples.

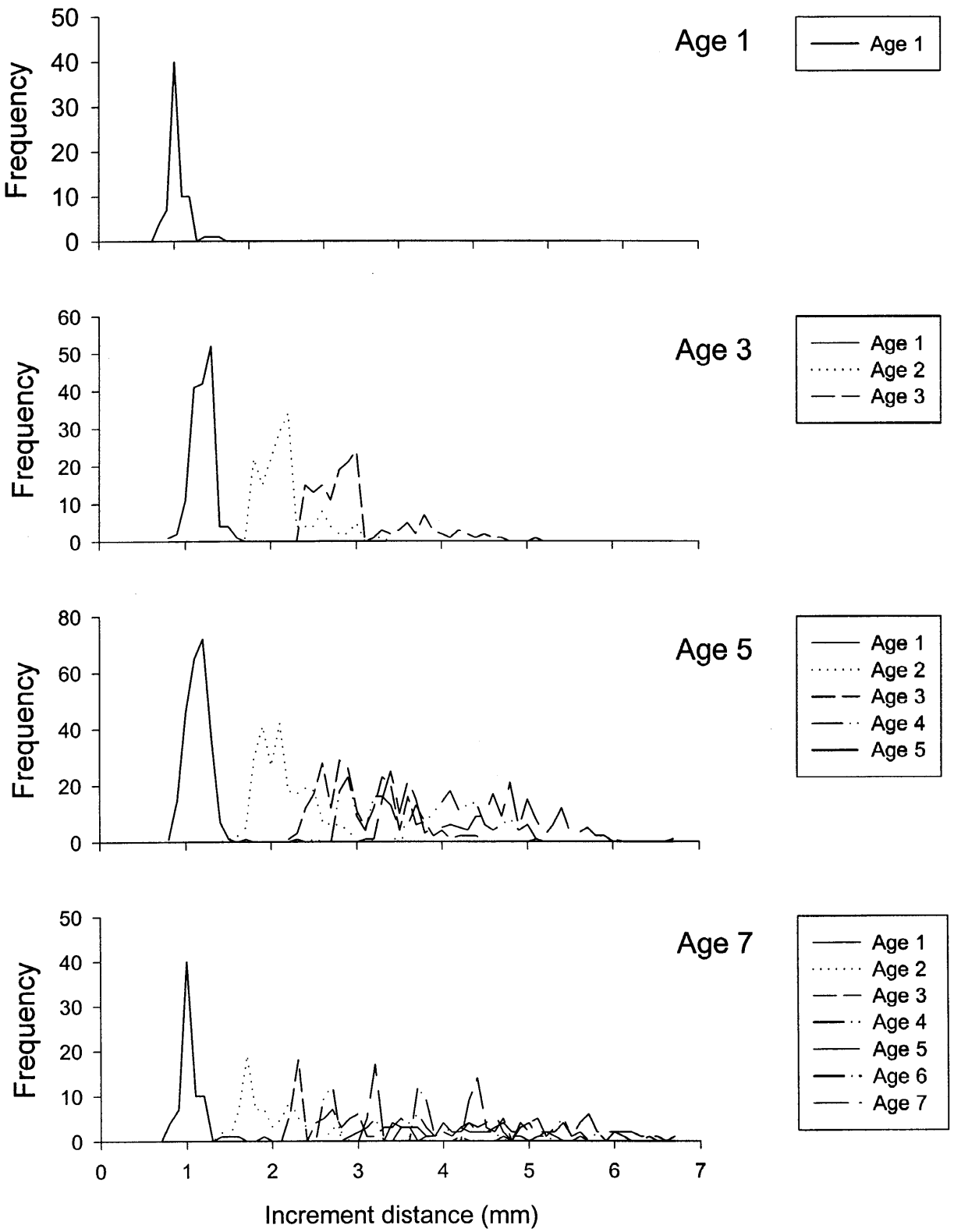

Age 1
Frequency
50
40
30
20
10
0
Age 1
Age 3
Frequency
60
50
40
30
20
10
0
Age 1
Age 2
Age 3
Age 5
Frequency
80
60
40
20
0
Age 1
Age 2
Age 3
Age 4
Age 5
Age 7
Frequency
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Increment distance (mm)
Age 1
Age 2
Age 3
Age 4
Age 5
Age 6
Age 7

Figure 9. Frequency of increment measurements for female gray triggerfish ages 1,3,5 and 7 from fishery-independent samples.

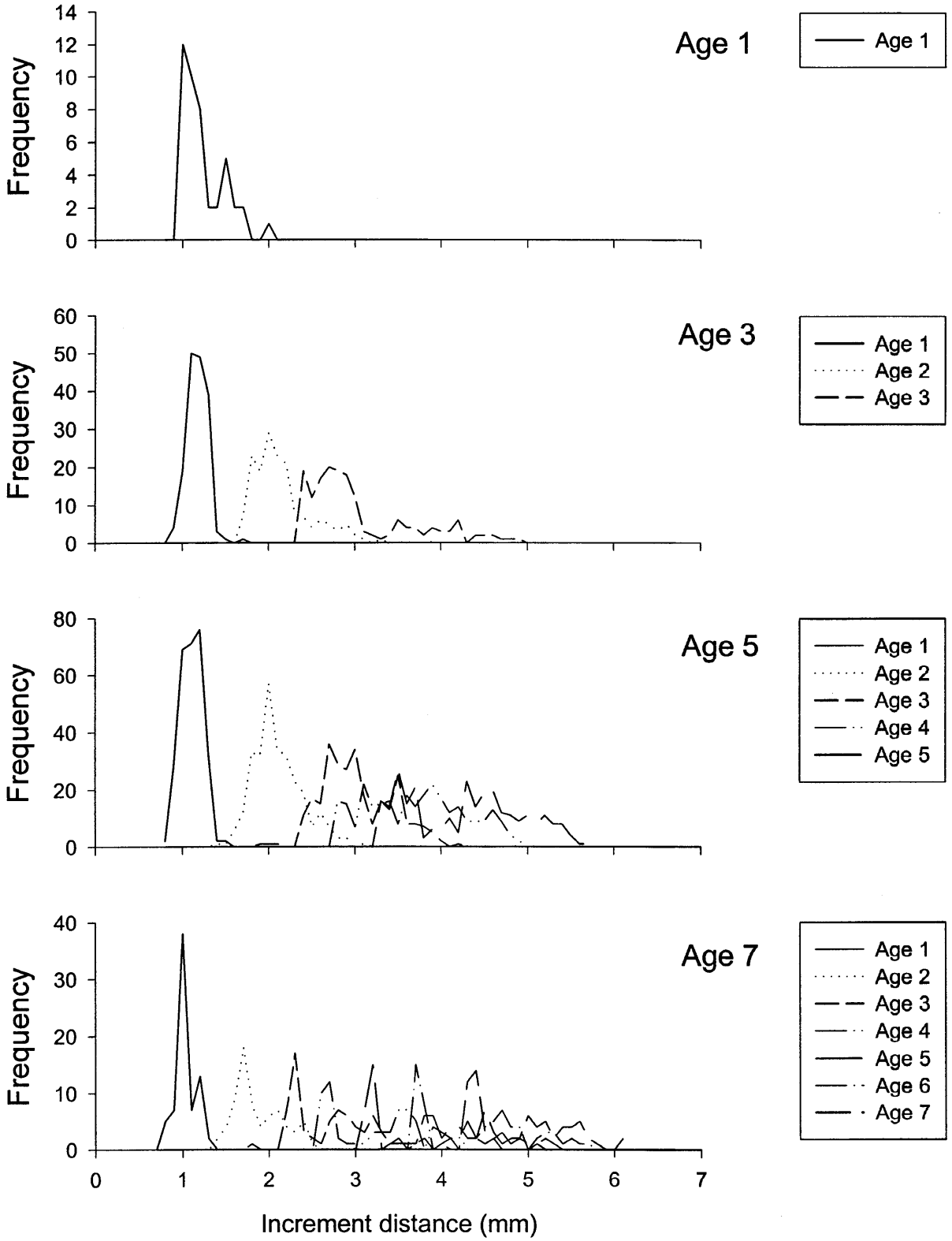

Age 1
Frequency
14
12
10
8
6
4
2
0
Age 1
Age 3
Frequency
60
50
40
30
20
10
0
Age 1
Age 2
Age 3
Age 5
Frequency
80
60
40
20
0
Age 1
Age 2
Age 3
Age 4
Age 5
Age 7
Frequency
40
30
20
10
0
Age 1
Age 2
Age 3
Age 4
Age 5
Age 6
Age 7
0
1
2
3
4
5
6
7
Increment distance (mm)

Figure 10. Observed and back-calculated length at age for male and female gray triggerfish from fishery-independent samples during 1992-1997. Error bars represent ±1 standard error (SE).

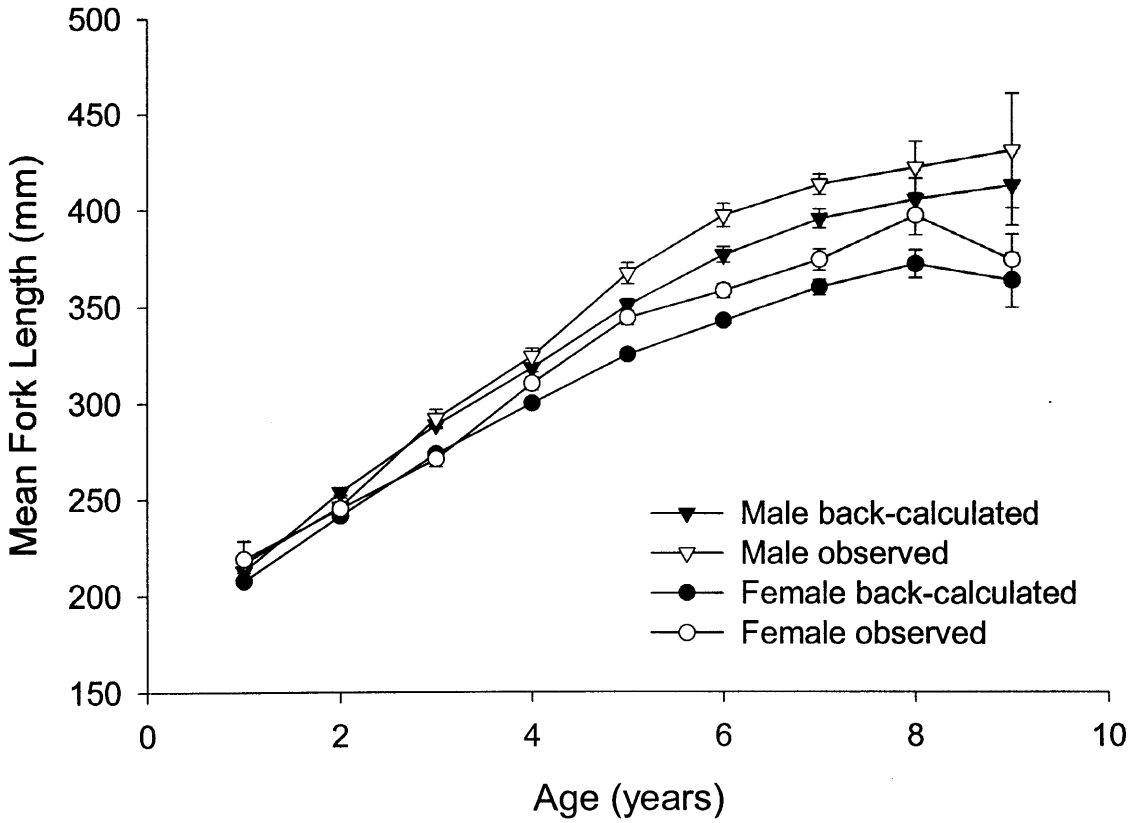

500
450
400
350
300
250
200
150
Mean Fork Length (mm)
0
2
4
6
8
10
Age (years)
Male back-calculated
Male observed
Female back-calculated
Female observed

Figure 11. Observed and back-calculated length at age for male and female gray triggerfish from fishery-dependent samples during 1992-1997. Error bars represent ±1 standard error (SE).

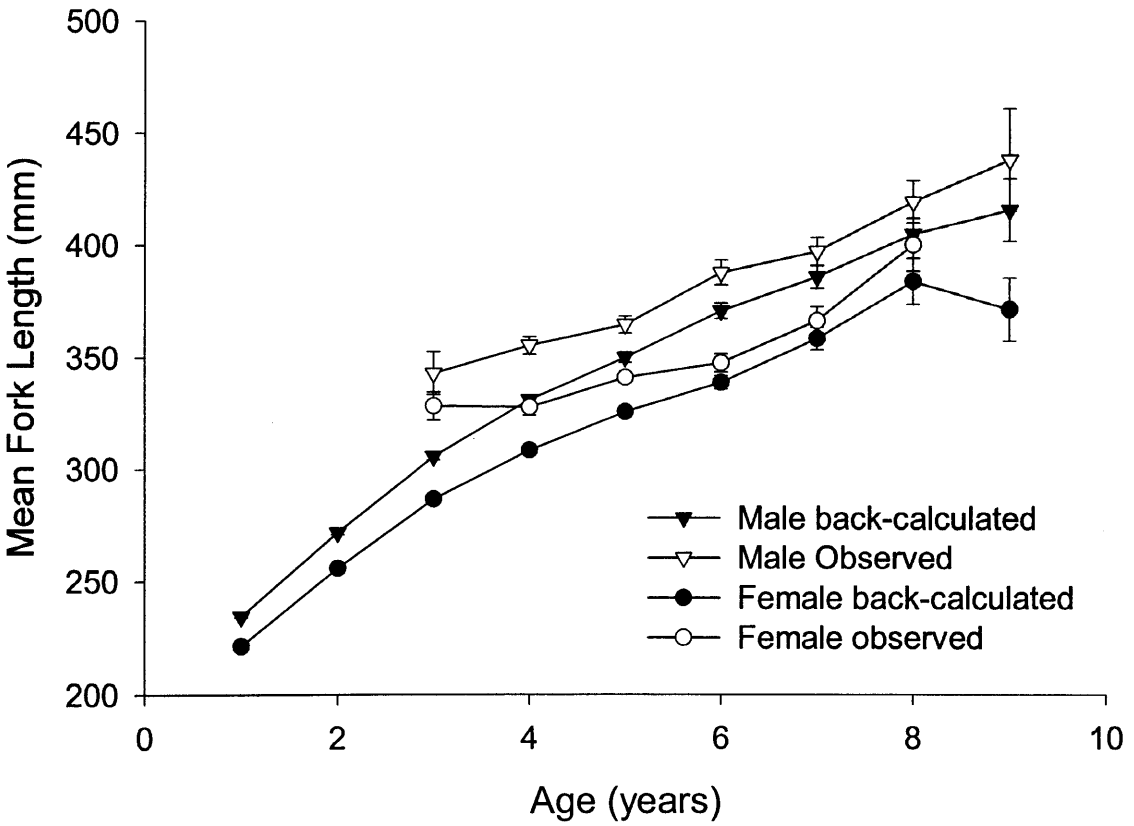

Mean Fork Length (mm)
500
450
400
350
300
250
200
0
2
4
6
8
10
Age (years)
Male back-calculated
Male Observed
Female back-calculated
Female observed

Figure 12. von Bertalanffy growth curves based on observed and back-calculated lengths at age for male and female gray triggerfish from fishery-independent samples during 1992-1997. Error bars represent ±1 standard error (SE).

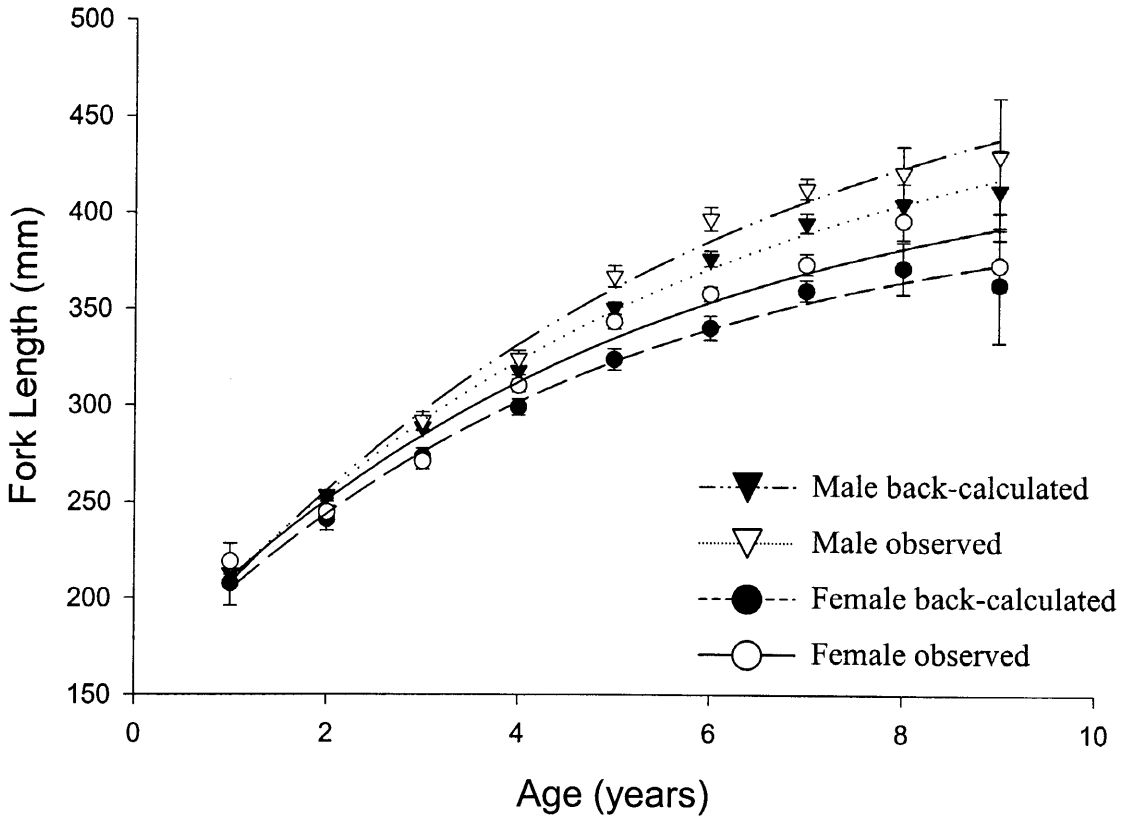

500
450
400
350
300
250
200
150
Fork Length (mm)
0
2
4
6
8
10
Age (years)
Male back-calculated
Male observed
Female back-calculated
Female observed

Figure 13. von Bertalanffy growth curves based on back-calculated lengths at age for male and female gray triggerfish from fishery-dependent samples during 1996-1997. Error bars represent ±1 standard error (SE).

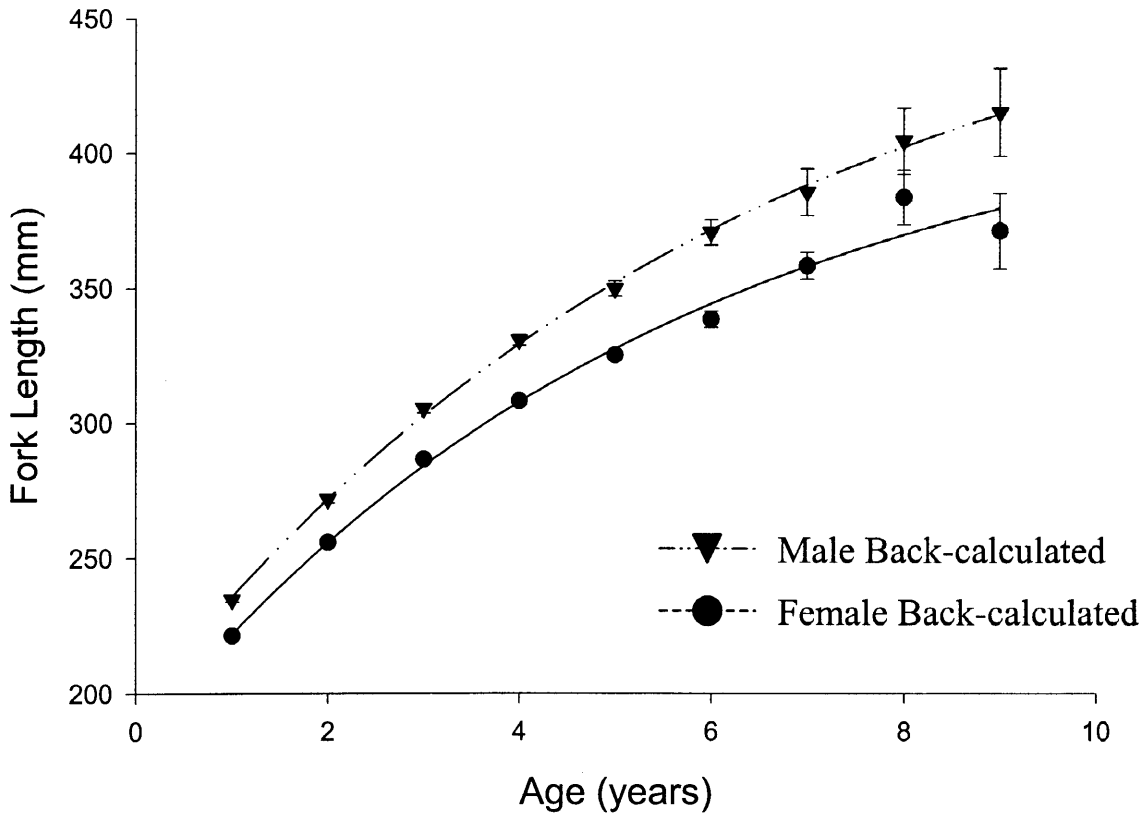

450
400
350
300
250
200
Fork Length (mm)
0
2
4
6
8
10
Age (years)
Male Back-calculated
Female Back-calculated

Figure 14. Observed and back-calculated growth rates for male and female gray gray triggerfish from fishery-independent samples. Error bars represent ±1 SE.

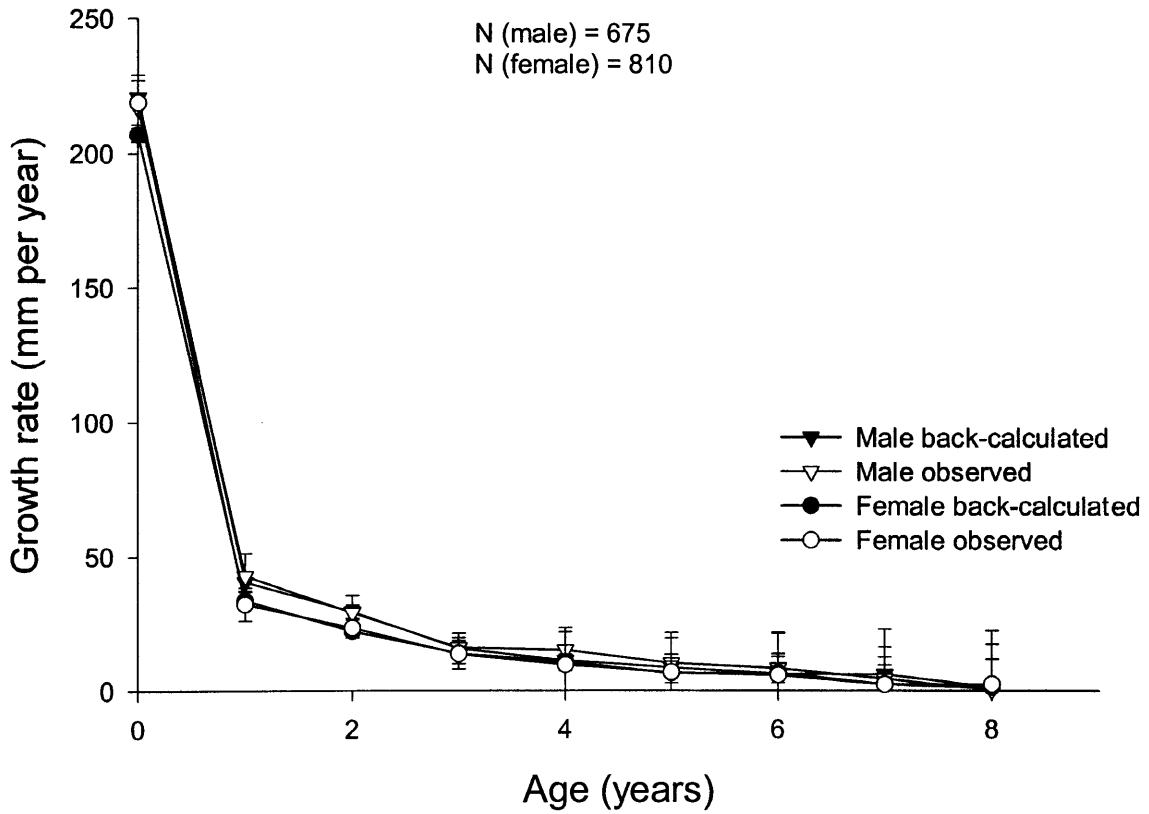

N (male) = 675
N (female) = 810
Growth rate (mm per year)
250
200
150
100
50
0
0
2
4
6
8
Age (years)
Male back-calculated
Male observed
Female back-calculated
Female observed

Figure 15. Observed and back-calculated growth rates for male and female gray gray triggerfish from fishery-dependent samples. Error bars represent ±1 SE.

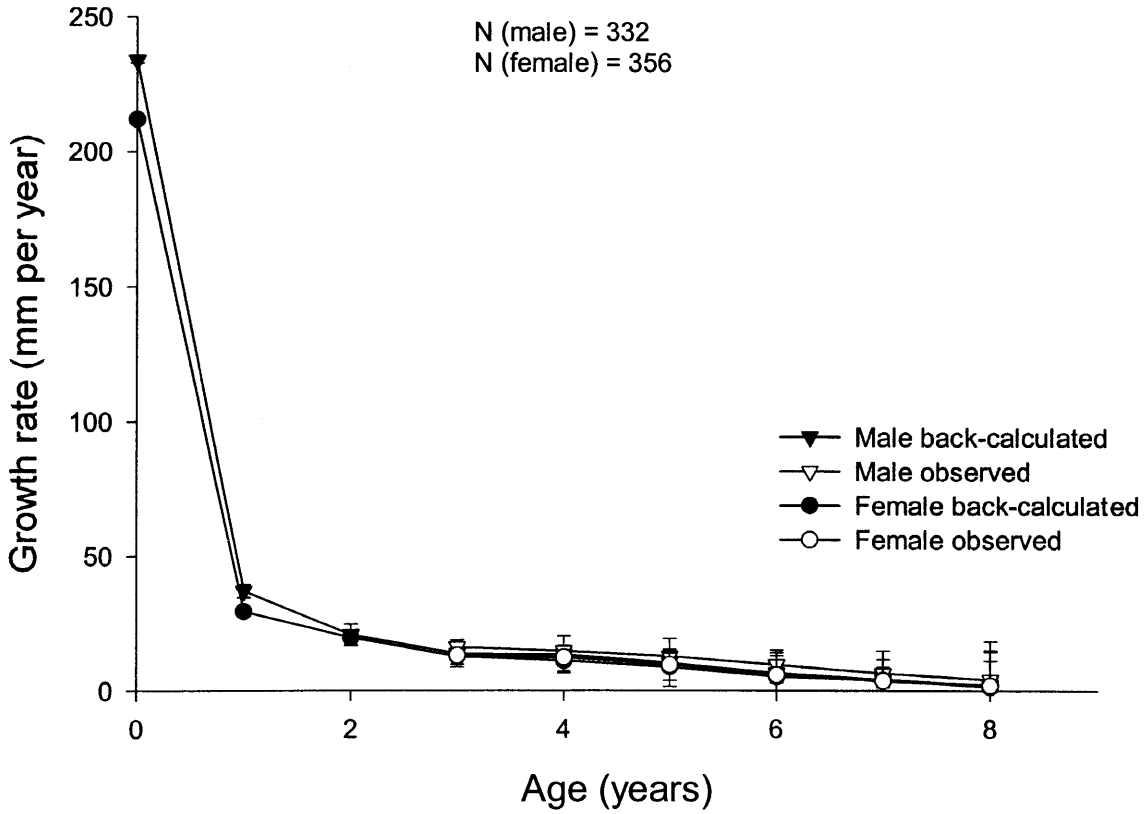
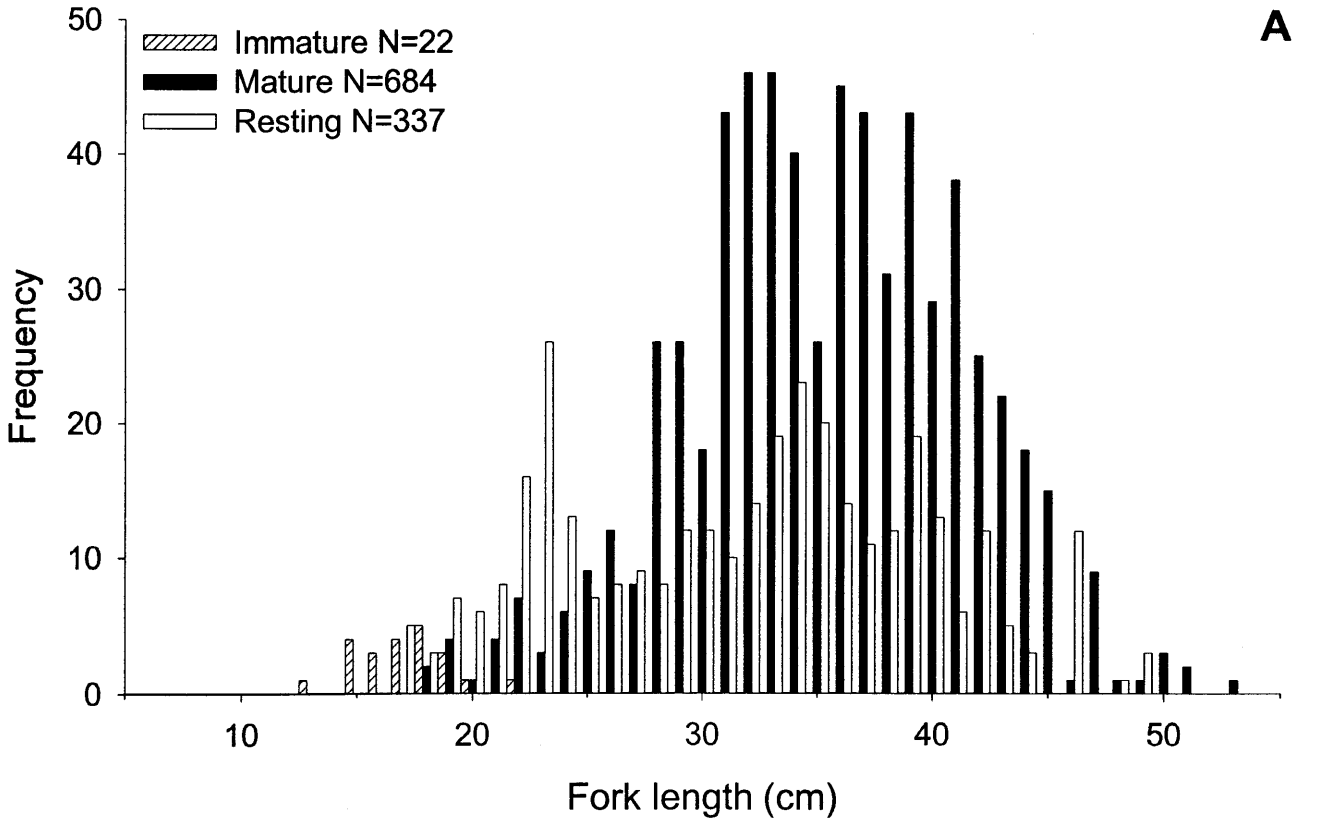

N (male) = 332
N (female) = 356
Growth rate (mm per year)
250
200
150
100
50
0
0
2
4
6
8
Age (years)
Male back-calculated
Male observed
Female back-calculated
Female observed

Figure 16. Length frequency histograms for immature, definitely mature and resting male (A) and female (B) gray triggerfish.


A
Immature N=22
Mature N=684
Resting N=337
Frequency
Fork length (cm)
0
10
20
30
40
50

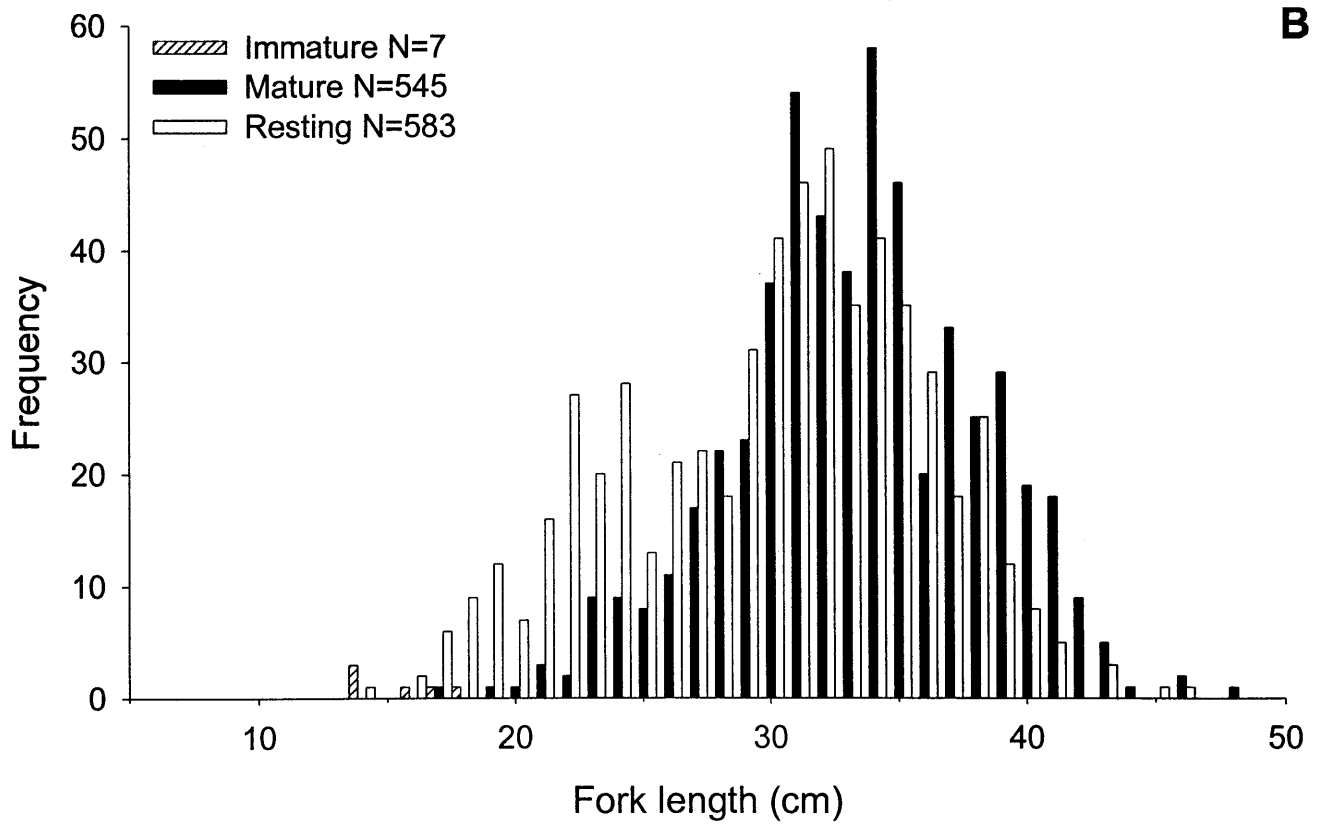
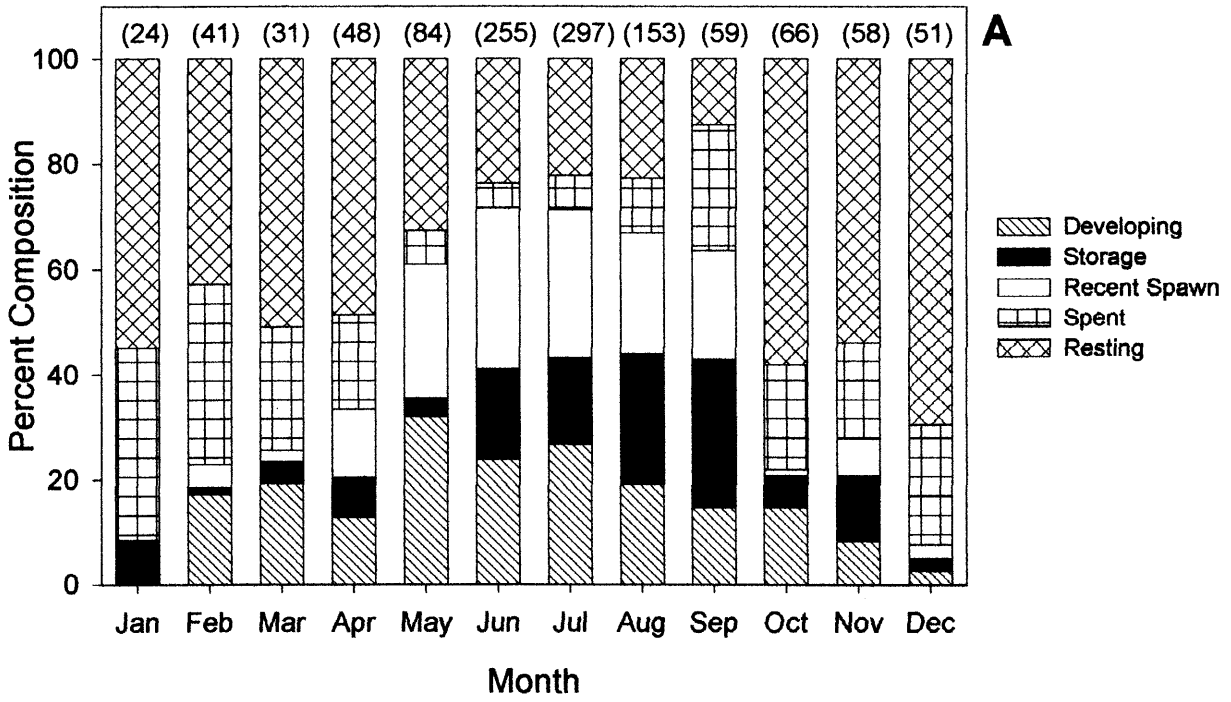

B
Immature N=7
Mature N=545
Resting N=583
Frequency
Fork length (cm)
0
10
20
30
40
50
60

Figure 17. Spawning season for male (A) and female (B) gray triggerfish during 1992-1997. Number above each bar represents sample size. POF= postovulatory follicle.


(24) (41) (31) (48) (84) (255) (297) (153) (59) (66) (58) (51)
A
Percent Composition
100
80
60
40
20
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Developing
Storage
Recent Spawn
Spent
Resting

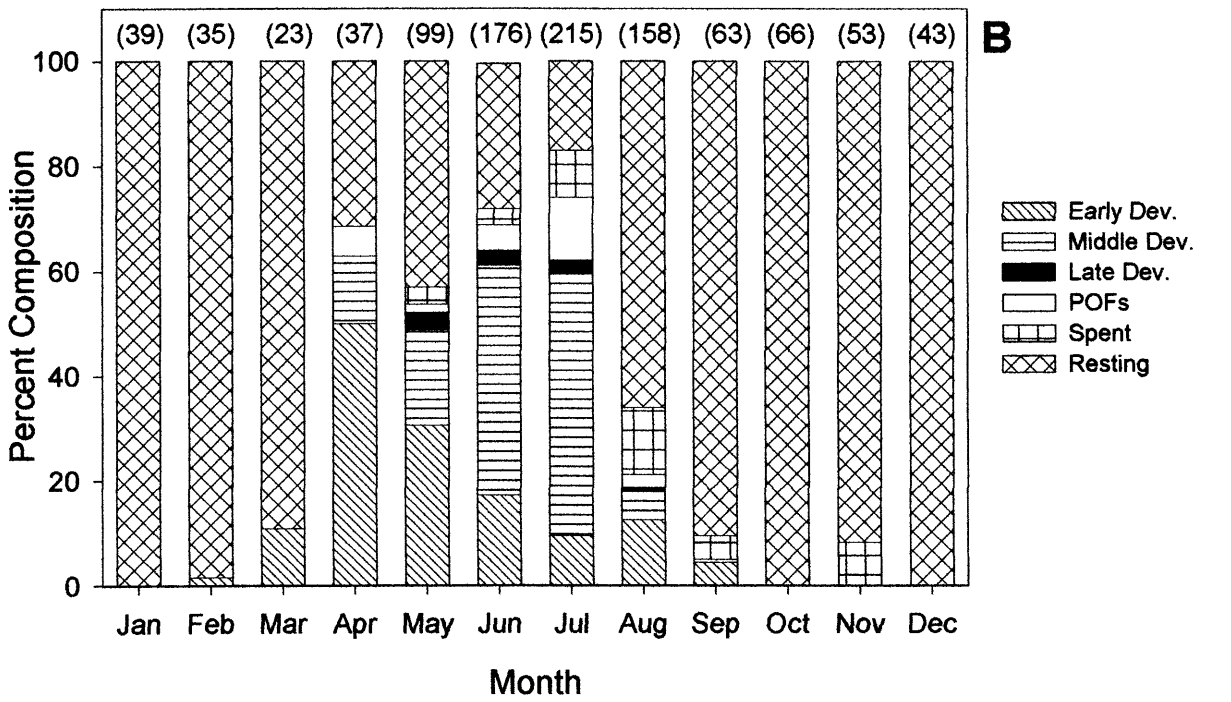

(39) (35) (23) (37) (99) (176) (215) (158) (63) (66) (53) (43)
B
Percent Composition
100
80
60
40
20
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
Early Dev.
Middle Dev.
Late Dev.
POFs
Spent
Resting

Figure 18. Mean gonadosomatic index (GSI) by month for female gray triggerfish from fishery-independent and fishery-dependent samples during 1996-1997. Sample size is in parentheses.

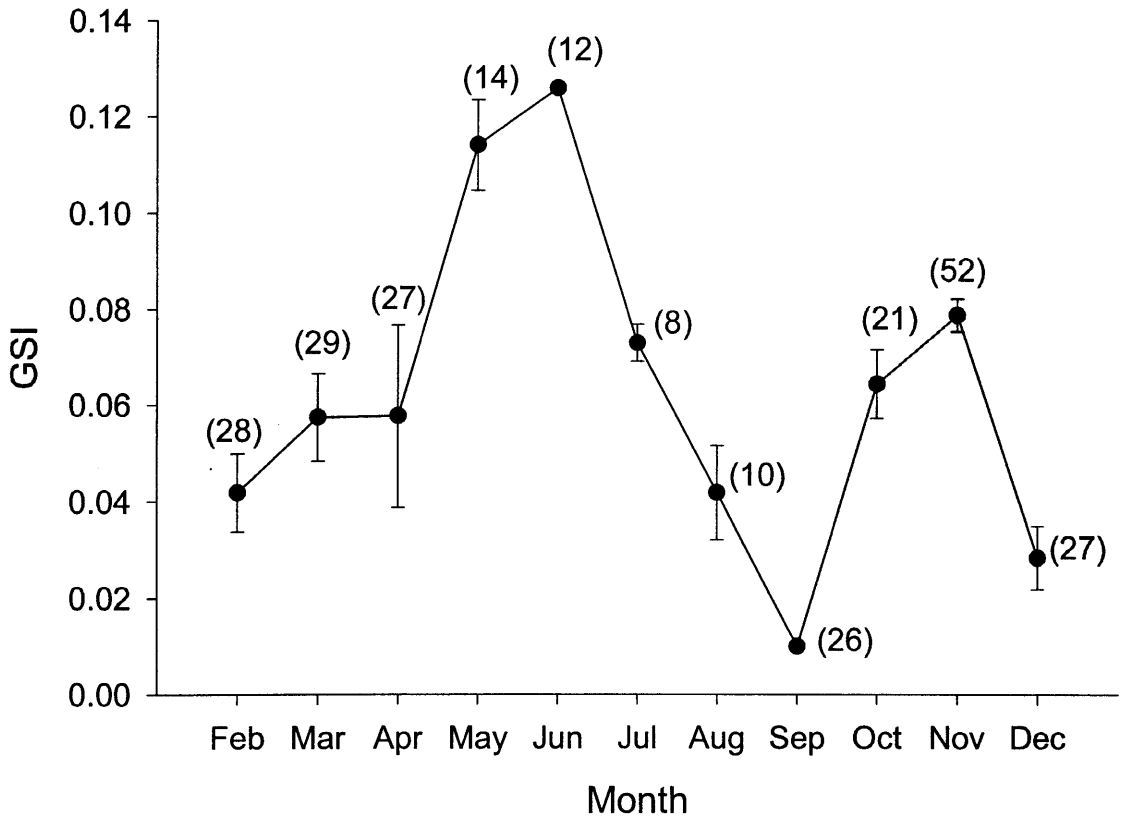
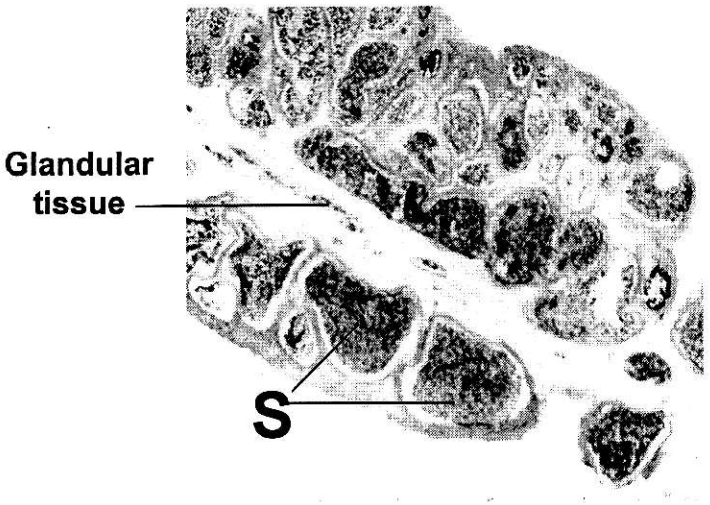
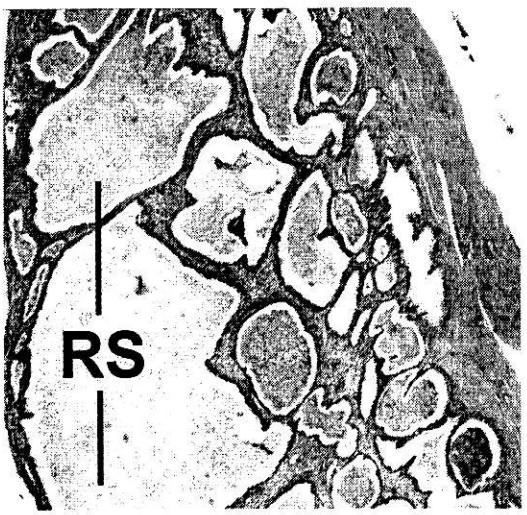

0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
GSI
(28)
(29)
(27)
(14)
(12)
(8)
(10)
(26)
(21)
(52)
(27)
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month

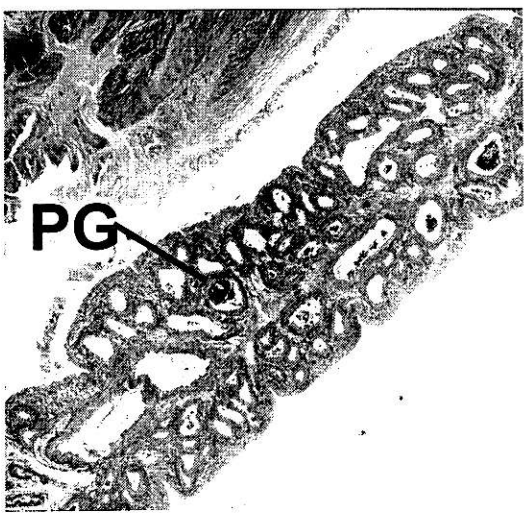
Figure 19. Gray triggerfish accessory gland ducts from the storage (A), recent spawn (B), and spent (C) stages of maturity. S = spermatozoa, RS = residual spermatozoa, PG = phagocytic granules. Magnification = 10X.



(A)

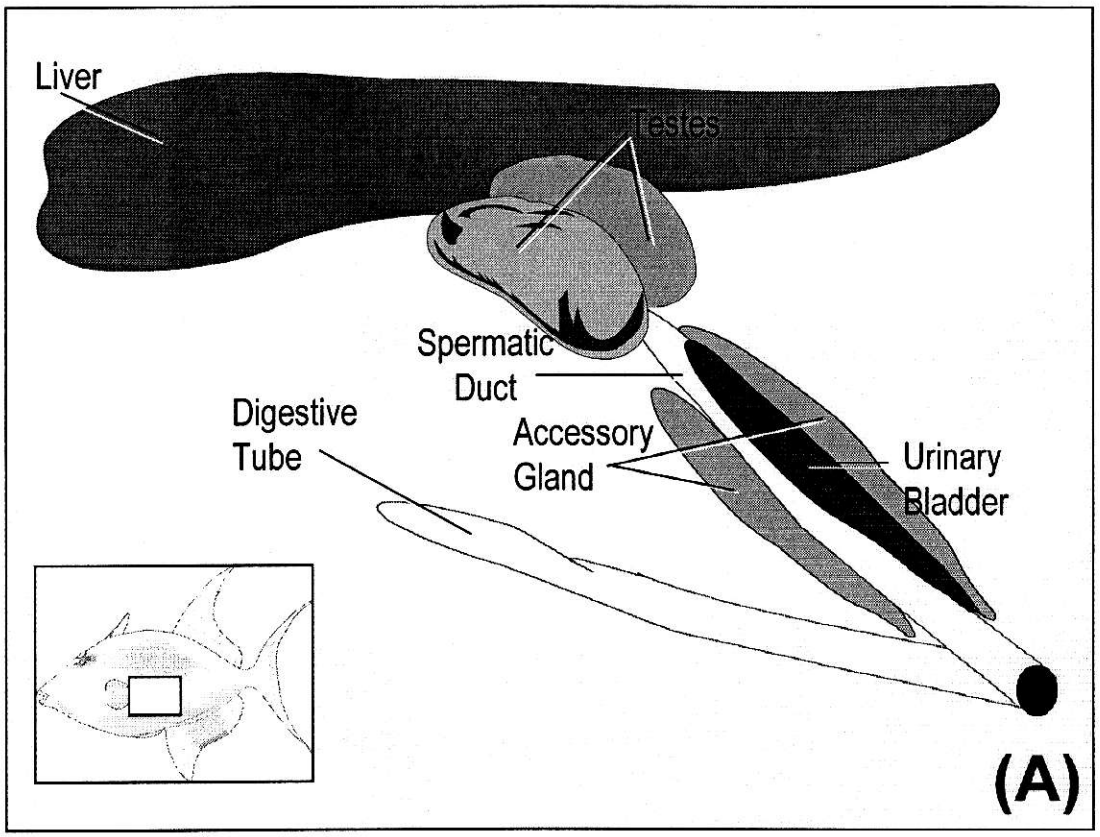


(B)



(C)

Figure 20. Illustration of the internal anatomy of the gray triggerfish depicting location of the testes within the body cavity.


Liver
Testes
Spermatic Duct
Digestive Tube
Accessory Gland
Urinary Bladder
(A)

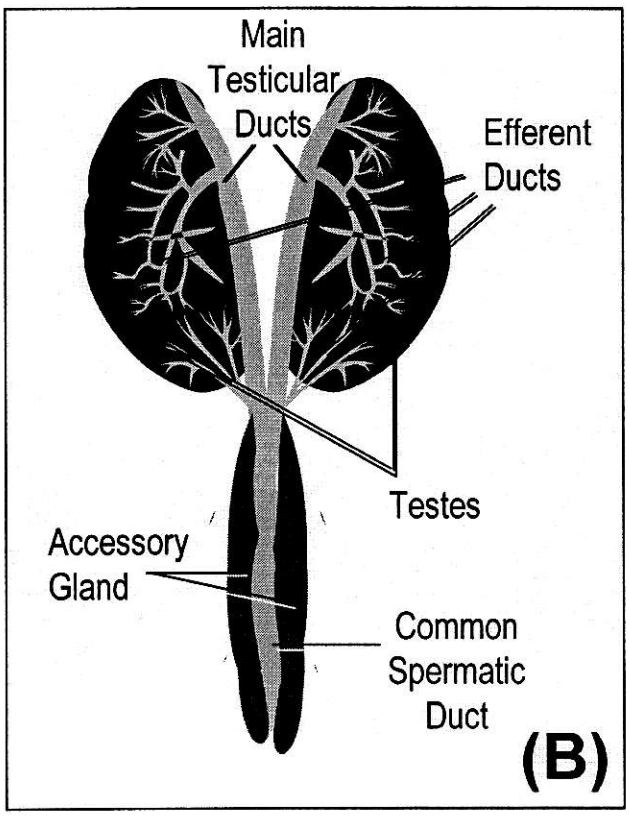

Main Testicular Ducts
Efferent Ducts
Testes
Accessory Gland
Common Spermatic Duct
(B)

Figure 21. Photograph of the spermatocyst within the gray triggerfish testis during the developing stage of maturity. Spermatozoa are darkly stained and located around the periphery of the cyst, with the orientation of spermatozoa heads normal to the wall.

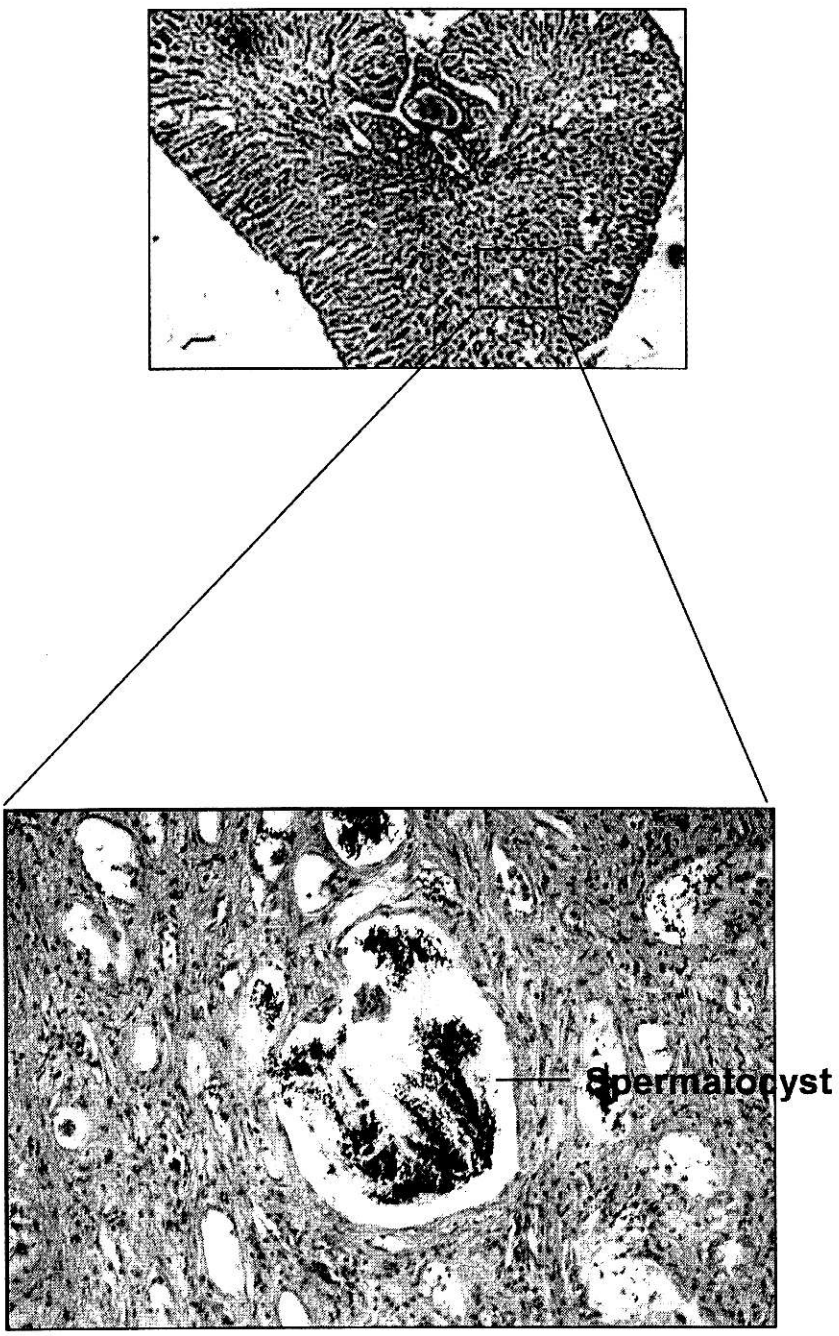
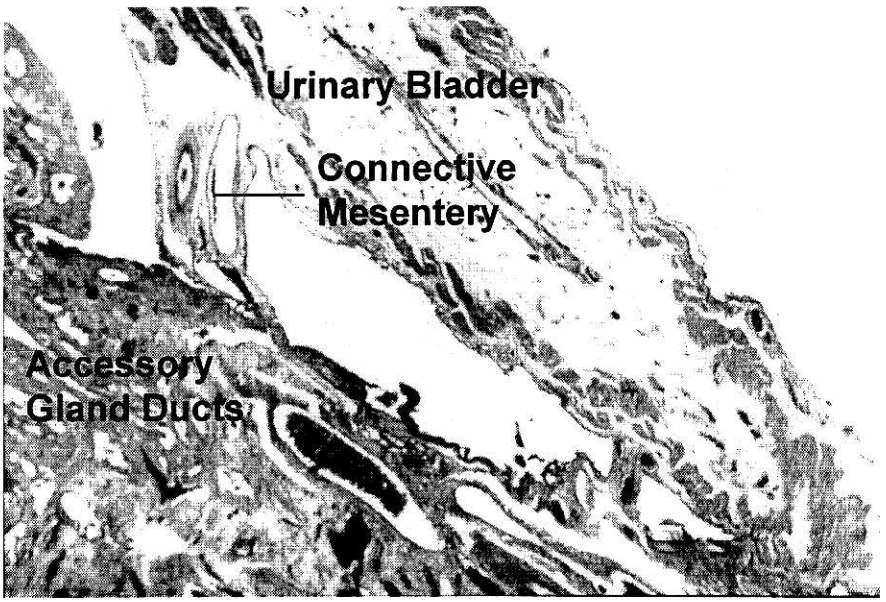
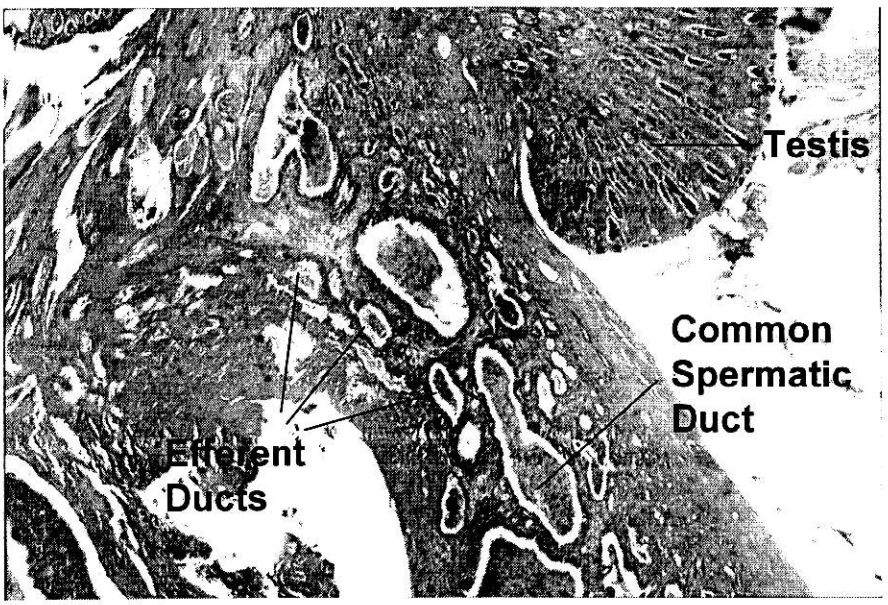

Spermatocyst

Figure 22. Longitudinal section of the male gonad, depicting the connectivity of the urinary bladder and the accessory gland ducts (A) and the close proximity of these structures (B).


Urinary Bladder
Connective Mesentery
Accessory Gland Ducts

(A)


Testis
Common Spermatic Duct
Efferent Ducts

(B)

Table 1. Histological criteria to assess reproductive state in male and female gray triggerfish, modified from Harris and McGovern (1997).

Reproductive State	Male	Female
Immature	Small cross-section, compared to resting male; little or no spermatocyte development	Primary growth oocytes (<60μm in diameter) only, no evidence of atresia. In comparison to resting female, transverse section of ovary is smaller, lamellai lack muscle and connective tissue bundles and are not as elongate, oogonia abundant along margin of lamellae, ovarian wall is thinner
Developing	Limited spermatogenesis, amount of steroidogenic tissue (adjacent to testicular duct) increases with size of male; elongation of lobules and some accumulation of spermatozoa in lobules and ducts	See below
Storage	Spermatic ducts are densely packed with spermatozoa; little or no spermatogenesis in testes	N/A
Recent Spawn	Residual spermatozoa in extremely enlarged ducts lined with columnar secretory cells; little or no spermatogenesis in testis	See "Developing, recent spawn" below
Spent	Compressed and collapsing ducts with little or no residual spermatozoa; no spermatogenesis evident in testis	>50% of vitellogenic oocytes undergoing atresia
Resting	Little residual spermatozoa in lobules, efferent ducts and main spermatic duct; some early spermatogenesis may be present in testis	Primary growth oocytes > 60 μm in diameter, with traces of atresia possible. In comparison to immature female, transverse section of ovary is larger, lamellae have muscle and connective tissue bundles and are more elongate and convoluted, oogonia less abundant along margin of lamellae, ovarian wall is thicker and exhibits varying degrees of expansion due to previous spawning
Early developing, cortical alveoli		Most advanced oocytes in cortical alveoli stage
Developing, vitellogenesis		Most advanced oocytes in yolk-globule stage
Late Developing, yolk coalescence		Most advanced oocytes in migratory-nucleus stage; partial coalescence of yolk globules possible
Developing, recent spawn (early)		Small vitellogenic or cortical alveoli stage oocytes predominant and presence of stage 1 postovulatory follicles (POFs)
Developing, recent spawn (middle)		Small vitellogenic or cortical alveoli stage oocytes predominant and presence of stage 2 POFs
Developing, recent spawn (late)		Small vitellogenic or cortical alveoli stage oocytes predominant and presence of stage 3 POFs

Table 2. Least squares linear regression relating total length (TL) in mm to fork length (FL) in mm for each sex and gear type from 1992-1997 (some fish did not have a TL recorded). An equation for fishery-dependent samples, sexes combined, is also listed.

Total Length Equations:		
$TL_{Fishery\text{-}independent\ males} = 1.20\ (FL) - 15.26$	$n = 669$	$r^2 = 0.97$
$TL_{Fishery\text{-}dependent\ males} = 1.26\ (FL) - 32.48$	$n = 320$	$r^2 = 0.91$
$TL_{Fishery\text{-}independent\ females} = 1.19\ (FL) - 9.98$	$n = 809$	$r^2 = 0.84$
$TL_{Fishery\text{-}dependent\ females} = 1.23\ (FL) - 25.38$	$n = 347$	$r^2 = 0.86$
$TL_{Fishery\text{-}dependent\ (sexes\ combined)} = 1.24\ (FL) - 28.86$	$n = 723$	$r^2 = 0.94$

Table 3. Least squares linear regression relating standard length (SL) in mm to fork length (FL) in mm for each sex and gear type from 1992-1997 (some fish did not have a TL recorded). An equation for fishery-dependent samples, sexes combined, is also listed.

Standard Length Equations:		
$SL_{Fishery\text{-}independent\ males} = 0.86\ (FL) - 8.6$	$n = 675$	$r^2 = 0.99$
$SL_{Fishery\text{-}dependent\ males} = 0.86\ (FL) - 15.2$	$n = 332$	$r^2 = 0.96$
$SL_{Fishery\text{-}independent\ females} = 0.86\ (FL) - 8.9$	$n = 810$	$r^2 = 0.98$
$SL_{Fishery\text{-}dependent\ females} = 0.84\ (FL) - 7.7$	$n = 356$	$r^2 = 0.92$
$SL_{Fishery\text{-}dependent\ (sexes\ combined)} = 0.85\ (FL) - 12.1$	$n = 688$	$r^2 = 0.95$

Table 4. Least squares linear regression relating total body weight (TBW) in grams to total length (TL) in mm for each sex and gear type from 1992-1997(some fish were not weighed). An equation for fishery-dependent samples, sexes combined, is also listed.

Weight/Length Equations:		
$TBW_{\text{Fishery-independent males}} = 7.45\ (FL) - 1501.52$	$n = 673$	$r^2 = 0.91$
$TBW_{\text{Fishery-dependent males}} = 10.34\ (FL) - 2639.46$	$n = 332$	$r^2 = 0.90$
$TBW_{\text{Fishery-independent females}} = 6.61\ (FL) - 1261.87$	$n = 810$	$r^2 = 0.90$
$TBW_{\text{Fishery-dependent females}} = 8.40\ (FL) - 1937.82$	$n = 356$	$r^2 = 0.87$
$TBW_{\text{Fishery-dependent (sexes combined)}} = 7.33\ (FL) + -1529.28$	$n = 747$	$r^2 = 0.82$

Table 5. Mean observed and mean back-calculated fork length (FL) (mm) for gray triggerfish from fishery-independent samples.

Male

Age	Number	Mean Obs Length	Back-Calculated Lengths at Age								
			1	2	3	4	5	6	7	8	9
1	31	217	207								
2	79	246	202	234							
3	141	286	212	244	273						
4	168	324	216	252	286	308					
5	135	367	216	269	300	326	351				
6	78	397	215	265	303	328	354	381			
7	30	413	215	258	292	318	345	371	398		
8	11	422	212	253	287	311	340	365	391	407	
9	2	431	214	238	265	291	325	353	378	394	413
		Mean	**212**	**252**	**287**	**314**	**343**	**368**	**389**	**401**	**413**
N	**675**		**675**	**644**	**565**	**424**	**256**	**121**	**43**	**13**	**2**

Female

Age	Number	Mean Obs Length	Back-Calculated Lengths at Age								
			1	2	3	4	5	6	7	8	9
1	44	219	207								
2	96	245	199	232							
3	150	271	204	234	260						
4	212	310	210	244	276	296					
5	145	344	212	250	284	308	330				
6	150	358	211	245	277	300	324	345			
7	38	374	208	240	271	295	317	338	360		
8	13	391	211	243	271	289	314	338	364	378	
9	4	374	204	233	256	275	300	322	341	353	364
		Mean	**207**	**240**	**271**	**294**	**317**	**336**	**355**	**365**	**364**
N	**852**		**852**	**808**	**712**	**562**	**350**	**205**	**55**	**17**	**4**

Table 6. Mean observed and mean back-calculated fork length (FL) (mm) at age for gray triggerfish from fishery-dependent samples.

Male

Age	Number	Mean Obs Length	Back-Calculated Lengths at Age									
			1	2	3	4	5	6	7	8	9	10
1	0	-	-									
2	0	-	-	-								
3	17	343	239	277	313							
4	84	355	235	274	311	336						
5	111	365	234	270	303	329	348					
6	66	388	235	272	306	332	354	372				
7	44	397	233	269	301	328	349	368	385			
8	7	419	238	265	295	322	352	375	392	406		
9	1	461	241	270	299	314	343	372	401	415	430	
10	1	415	235	261	287	300	325	351	376	389	402	403
		Mean	**236**	**270**	**302**	**323**	**345**	**368**	**388**	**403**	**416**	**403**
N	**331**		**331**	**331**	**331**	**314**	**230**	**119**	**53**	**9**	**2**	**1**

Female

Age	Number	Mean Obs Length	Back-Calculated Lengths at Age								
			1	2	3	4	5	6	7	8	9
1		-	-								
2		-	-	-							
3	16	327	222	268	290						
4	89	328	221	257	289	311					
5	138	341	221	255	285	309	327				
6	69	348	220	253	281	302	320	335			
7	33	366	221	252	283	306	326	341	354		
8	8	400	230	276	308	327	349	363	377	389	
9	2	379	232	265	284	297	312	327	351	363	371
		Mean	**224**	**261**	**288**	**309**	**327**	**341**	**361**	**376**	**371**
N	**355**		**355**	**355**	**355**	**339**	**250**	**112**	**43**	**10**	**2**

Table 7. Parameters of the von Bertalanffy growth curves fitted to the observed fork length (FL) from 1992-1997 for ages 1-10

	Males	Females
Fishery-independent		
L_∞	521.08	442.77
k	0.17	0.19
t_0	-2.03	-2.26

Table 8. Parameters of the von Bertalanffy growth curves fitted to the back-calculated fork length (FL) from 1992-1997 for ages 1-10

	Males	Females
Fishery-independent		
L_∞	486.24	412.54
k	0.18	0.21
t_0	-2.23	-2.25
Fishery-dependent		
L_∞	488.68	430.04
k	0.15	0.18
t_0	-3.27	-3.09

Table 9. Results of t-test for mean lengths at each age class between depth zones for fishery-independent samples. Shallow zone is <45 m, Deep zone is ≥ 45 m. Mean length at age for each depth zone is shown. Dash lines indicate insufficient data for comparison (α=0.05).

Age	Males			Females		
	Shallow	**Deep**	**Probability**	**Shallow**	**Deep**	**Probability**
1	211.37	-	N/A	215.23	-	N/A
2	242.64	356.00	<0.001	244.75	-	N/A
3	274.23	355.95	<0.001	259.72	325.03	<0.001
4	305.85	356.70	<0.001	283.44	338.50	<0.001
5	342.21	384.23	<0.001	316.028	352.28	<0.001
6	370.75	406.55	0.005	348.31	360.05	0.12
***7+**	434.5	414.43	0.12	338.5	382.67	0.006

*7+ is a compilation of all ages ≥7

Table 10. Age-length key for gray triggerfish caught with fishery-independent gear.

FL (cm)	Number	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9
		%	%	%	%	%	%	%	%	%	%
14	3	-	100.0	-	-	-	-	-	-	-	-
15	7	-	57.1	42.9	-	-	-	-	-	-	-
16	7	-	85.7	14.3	-	-	-	-	-	-	-
17	17	-	35.3	52.9	11.8	-	-	-	-	-	-
18	20	5.0	50.0	35.0	10.0	-	-	-	-	-	-
19	32	3.1	28.1	31.3	18.8	12.5	6.3	-	-	-	-
20	26	-	26.9	34.6	26.9	7.7	3.8	-	-	-	-
21	29	-	3.4	41.4	44.8	3.4	6.9	-	-	-	-
22	53	-	18.9	28.3	41.5	9.4	1.9	-	-	-	-
23	44	-	13.6	27.3	40.9	15.9	2.3	-	-	-	-
24	74	-	2.7	28.4	40.5	25.7	2.7	-	-	-	-
25	40	-	-	32.5	42.5	22.5	-	2.5	-	-	-
26	43	-	11.6	34.9	25.6	20.9	7.0	-	-	-	-
27	49	-	4.1	16.3	34.7	36.7	6.1	2.0	-	-	-
28	65	-	-	13.8	32.3	43.1	3.1	7.7	-	-	-
29	70	-	-	17.1	25.7	31.4	21.4	4.3	-	-	-
30	72	-	-	8.3	25.0	36.1	25.0	5.6	-	-	-
31	99	-	-	8.1	22.2	41.4	18.2	8.1	2.0	-	-
32	81	-	-	4.9	16.0	50.6	19.8	7.4	-	1.2	-
33	76	-	-	2.5	13.3	42.2	27.4	11.9	2.6	-	-
34	76	-	-	3.9	21.1	31.6	23.7	13.2	5.3	1.3	-
35	74	-	-	1.4	8.1	25.7	28.4	23.0	8.1	4.1	1.4
36	71	-	-	1.4	11.3	15.5	36.6	29.6	4.2	-	1.4
37	50	-	-	-	4.0	28.0	34.0	20.0	12.0	2.0	-
38	60	-	-	-	5.0	28.3	28.3	23.3	10.0	3.3	1.7
39	51	-	-	-	9.8	21.6	33.3	19.6	9.8	5.9	-
40	55	-	-	-	-	18.2	30.9	27.3	12.7	9.1	1.8
41	47	-	-	-	6.4	17.0	31.9	17.0	21.3	4.3	2.1
42	35	-	-	-	-	11.4	28.6	40.0	14.3	2.9	2.9
43	23	-	-	-	-	17.4	26.1	26.1	17.4	13.0	-
44	18	-	-	-	-	16.7	16.7	44.4	22.2	-	-
45	14	-	-	-	-	7.1	42.9	28.6	21.4	-	-
46	8	-	-	-	-	12.5	12.5	50.0	12.5	-	12.5
47	10	-	-	-	-	-	30.0	20.0	10.0	40.0	-
48	6	-	-	-	-	-	50.0	33.3	-	16.7	-
49	2	-	-	-	-	-	50.0	50.0	-	-	-
*50+	7	-	-	-	-	-	71.4	28.6	-	-	-
Total	**1514**										

*50+ is a compilation of all fork lengths (FL) >50 cm

Table 11. Age-length key for gray triggerfish caught with fishery-dependent gear.

FL (cm)	Number	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10
	%	%	%	%	%	%	%	%	%
27	3	-	66.7	-	33.3	-	-	-	-
28	8	12.5	62.5	25.0	-	-	-	-	-
29	14	21.4	35.7	28.6	14.3	-	-	-	-
30	22	18.2	63.6	18.2	-	-	-	-	-
31	53	5.7	43.4	37.7	7.5	5.7	-	-	-
32	74	5.4	27.0	44.5	18.9	4.1	-	-	-
33	70	2.9	31.4	40.0	18.6	7.1	-	-	-
34	86	2.3	25.6	50.0	17.4	4.7	-	-	-
35	68	8.8	25.0	38.2	25.0	2.9	-	-	-
36	65	9.2	16.9	41.5	21.5	7.7	1.5	1.5	-
37	46	2.2	28.3	32.6	17.4	19.6	-	-	-
38	42	2.5	9.5	33.4	23.8	22.5	8.2	-	-
39	39	-	12.8	28.2	33.3	23.1	2.6	-	-
40	38	2.6	18.4	36.8	13.2	21.1	5.3	2.6	-
41	24	-	29.2	29.2	12.5	25.0	4.2	-	-
42	21	-	9.5	38.1	23.8	14.3	9.5	-	4.8
43	21	-	9.5	14.3	38.1	23.8	14.3	-	-
44	8	-	25.0	25.0	50.0	0.0	-	-	-
45	5	-	20.0	40.0	0.0	40.0	-	-	-
46	8	-	12.5	12.5	12.5	25.0	25.0	12.5	-
47	5	-	-	20.0	60.0	20.0	-	-	-
48	1	-	-	-	100.0	0.0	-	-	-
49	2	-	-	-	50.0	50.0	-	-	-
*50+	3	-	-	-	-	33.3	66.7	-	-
Total	**726**								

*50+ is a compilation of all fork lengths (FL) >50 cm

Table 12. Chi-Square analyses of sex ratio for gray triggerfish from fishery-independent samples. H_0:Male to female ratio is 1:1

Fork Length (mm)	Males	Females	Female:Male	Probability	H_0
<175	17	17	1:1	1.00	Accept
176-200	29	40	1.38:1	0.19	Accept
210-225	36	50	1.39:1	0.13	Accept
226-250	60	79	1.32:1	0.11	Accept
251-275	42	66	1.57:1	0.02	Reject
276-300	75	92	1.23:1	0.19	Accept
301-325	84	135	1.61:1	<0.01	Reject
326-350	73	118	1.62:1	<0.01	Reject
351-375	68	88	1.29:1	0.06	Accept
376-400	59	70	1.19:1	0.33	Accept
401-425	61	40	0.66:1	0.04	Reject
426-450	36	12	0.33:1	<0.01	Reject
451-475	22	1	0.05:1	<0.01	Reject
476<	13	2	0.15:1	<0.01	Reject
Overall	**675**	**810**	**1.2:1**	**<0.01**	**Reject**

Table 13. Chi-Square analyses of sex ratio for gray triggerfish from fishery-dependent samples. H_0: Male to female ratio is 1:1

Fork Length (mm)	Males	Females	Female:Male	Probability	H_0
<300	8	29	3.63:1	0.01	Reject
301-325	36	98	2.72:1	<0.01	Reject
326-350	84	98	1.17:1	0.30	Accept
351-375	62	72	1.16:1	0.39	Accept
376-400	58	40	0.69:1	0.07	Accept
401-425	44	11	0.25:1	<0.01	Reject
426-450	24	4	0.17:1	<0.01	Reject
451<	15	4	0.27:1	0.01	Reject
Overall	**331**	**356**	**1.08:1**	**0.34**	**Accept**

Table 14. Percentage of mature female specimens by size and age class for gray triggerfish from fishery-dependent samples

FL (mm)	Age 0		Age 1		Age 2		Age 3		Age 4		Age 5		Age 6		Age 7+		Total	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
<175	0	(2)	60	(5)	50	(4)											45	(11)
176-200			94	(17)	100	(6)	100	(6)	100	(1)							97	(30)
201-225			100	(8)	100	(14)	100	(20)	100	(2)	100	(3)					100	(47)
226-250			100	(2)	100	(20)	100	(34)	100	(21)							100	(77)
251-275			100	(6)	100	(21)	100	(17)	100	(19)	100	(2)	100	(1)			100	(66)
276-300					100	(12)	100	(27)	100	(33)	100	(15)	100	(4)			100	(91)
301-325					100	(5)	100	(20)	100	(60)	100	(30)	100	(16)	100	(2)	100	(133)
326-350					100	(1)	100	(13)	100	(41)	100	(28)	100	(23)	100	(12)	100	(118)
351-375							100	(6)	100	(13)	100	(33)	100	(26)	100	(9)	100	(87)
376-400					100	(1)	100	(1)	100	(12)	100	(21)	100	(17)	100	(16)	100	(68)
401-425							100	(2)	100	(5)	100	(9)	100	(14)	100	(10)	100	(40)
>426									100	(3)	100	(3)	100	(2)	100	(6)	100	(14)
Total	**0%**	**(2)**	**98%**	**(38)**	**100%**	**(84)**	**100%**	**(146)**	**100%**	**(210)**	**100%**	**(144)**	**100%**	**(103)**	**100%**	**(37)**	**Total**	**782**

Table 15. Percentage of mature male specimens by size and age class for gray triggerfish from fishery-dependent samples

FL (mm)	Age 0		Age 1		Age 2		Age 3		Age 4		Age 5		Age 6		Age 7+		Total	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
<175			22	(9)	43	(7)	0	(1)									47	(17)
176-200			63	(8)	82	(11)	67	(3)	50	(2)	50	(2)					69	(26)
201-225			100	(4)	90	(10)	100	(17)	100	(4)							97	(35)
226-250			100	(5)	100	(18)	100	(24)	100	(10)	100	(2)	100	(1)			100	(60)
251-275			100	(1)	100	(5)	100	(17)	100	(13)	100	(4)					100	(40)
276-300					100	(11)	100	(22)	100	(27)	100	(11)	100	(6)			100	(77)
301-325					100	(8)	100	(21)	100	(35)	100	(17)	100	(2)			100	(93)
326-350					100	(3)	100	(16)	100	(25)	100	(20)	100	(6)	100	(2)	100	(72)
351-375					100	(1)	100	(7)	100	(22)	100	(18)	100	(15)	100	(4)	100	(64)
376-400					100	(1)	100	(6)	100	(17)	100	(19)	100	(8)	100	(8)	100	(59)
401-425							100	(2)	100	(7)	100	(20)	100	(17)	100	(14)	100	(60)
426-450							100	(3)	100	(5)	100	(9)	100	(12)	100	(7)	100	(36)
Total			**63%**	**27**	**91%**	**75**	**98%**	**139**	**99%**	**168**	**99%**	**135**	**100%**	**79**	**100%**	**43**	**Total**	**673**