





Article

Age, Growth, and Functional Gonochorism with a Twist of Diandric Protogyny in Goliath Grouper from the Atlantic Coast of Florida

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Abstract: The lack of data on the age, growth, and reproduction of the Atlantic Goliath Grouper *Epinephelus itajara* off the coasts of Florida (USA) makes it difficult to estimate the extent of their population recovery following the 1990 fishery closure, as well as the potential effects of the recent (2023) opening of a fishery for juveniles. Goliath Grouper from the Atlantic coast of Florida were non-lethally sampled for size (total length, TL), age (via fin rays), and sex and reproductive stage (via gonad biopsies, milt/egg expression, gonopores) from May to October 2010–2016. Of 653 unique fish captured (not including 118 recaptures), 257 (39.4%) were females (122–228 cm TL; 5–20 years old), 264 (40.0%) were males (104–225 cm TL; 4–22 years old), 100 (15.3%) were unsexed (82–211 cm TL; 3–15 years old), and 32 (4.9%) were protogynous hermaphrodites (108–209 cm TL; 5–20 years old). Protogyny was conclusively determined in two fish recaptured and re-biopsied that had transitioned from female to male. However, an overlap in the age and size of males and females, a 1:1 sex ratio, and the presence of relatively small, young, mature males, in combination with an apparently low functional sex change rate, all indicated that the sexual pattern of Goliath Grouper was functionally gonochoristic with the potential for diandric protogyny. Females > 10 years old were larger-at-age than males, and Goliath Grouper from the Atlantic coast were larger-at-age than fish from the Gulf of Mexico. These differences in age, growth, and reproductive strategy—as well as the nascent fishery for juveniles—need to be monitored closely so that the current and future reproductive capacity of the population continues to ensure growth and sustainability.

Keywords: *Epinephelus itajara*; Goliath Grouper; functionally gonochoristic; diandric protogynous hermaphrodite; non-lethal ageing; transitional fish; gonad biopsies; reproductive classification

Key Contribution: Conclusively for the first time, Goliath Grouper in the Southeastern USA Atlantic were shown to be protogynous hermaphrodites and, most probably, exhibit diandric protogyny. However, their sexual pattern appears to be functionally gonochoristic, based on their size, age, and sex ratio demographics in combination with the low rate of known functional sex change. Goliath Grouper from the Atlantic coast of Florida are also sexually dimorphic, with females larger-at-age than males, and overall are larger-at-age compared to fish in the Gulf of Mexico.



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1. Introduction

Worldwide, warm-temperate and tropical groupers (family Epinephelidae) are heavily exploited, necessitating restrictive fishing regulations or complete protection [1]. Large grouper species, in particular, are vulnerable to over-exploitation because of their combined high economic value, slow growth, long life span, and late age and large size at sexual maturity. Groupers also tend to have high site fidelity and complex reproductive biology and many species form easily exploited spawning aggregations [1–6]. Atlantic Goliath Grouper *Epinephelus itajara* (hereafter referred to as Goliath Grouper) exemplify this vulnerability. They are the largest grouper found in coastal regions of the western Atlantic Ocean, with adults reaching 2.5 m in total length (TL) [7] and ages up to at least 37 years [8]. In addition, they form spawning aggregations during specific times of the year at specific sites [9,10], making them targets for harvesting [11]. Goliath Grouper also exhibit an ontogenetic shift in preferred habitat, with juveniles (≤ 6 years old but up to ~ 1.2 m TL) occurring mostly in coastal mangrove habitats and subadults and adults moving to relatively shallow (< 50 m) offshore reefs, ledges, and artificial structures [3,12,13].

A moratorium on fishing for Goliath Grouper was enacted in 1990 in the United States due to severe overfishing [14,15]. In 1996, the International Union for the Conservation of Nature (IUCN) listed the species globally as “critically endangered” [16], a status that continued until 2018, when their status was changed to “vulnerable” [17]. With the exception of the USA, harvest closures and fishery monitoring in other countries throughout the Goliath Grouper’s range have been met with limited success [17–20]. After over three decades of full protection from harvest in the Southeastern USA, however, an appreciable increase in Goliath Grouper abundance has been noted along both coasts of Florida [12,21–24]. This has led to considerable debate as to whether or not the increase in abundance has been sufficient to allow the re-establishment of a limited fishery, or if the economic gain derived from ecotourism surrounding the iconic Goliath Grouper outweighs a take fishery [20,25]. Despite a limited understanding of the current population demographics, a restricted slot-based fishery for juveniles (200 individuals between 24 and 36 inches (61.0 to 91.5 cm) TL) was initiated in Florida in the spring of 2023.

While population assessments across juvenile and adult life stages are critical in addressing issues related to Goliath Grouper’s over-exploitation and recovery status [26], conventional data collection through fishery-dependent means, including biological data, has not been available since 1990. Bullock et al. [8] sampled commercial and recreational catches of Goliath Grouper from 1977 to 1990 in the Gulf of Mexico (GOM) and provided the most comprehensive age, growth, and reproductive study of Goliath Grouper available to date. Within the past 15 years, however, substantial information on the relative abundance, movements, spawning behavior, habitat use, diet, and mercury contamination have been amassed using non-lethal collection methods [10,13,23,27–31]. These studies have been particularly important in determining the spatiotemporal use of nursery and spawning sites [10,32], with related movements and spawning behavior both in the GOM and off the Atlantic coast of Florida [10,28,32].

Understanding the reproductive biology of Goliath Grouper in relation to spawning is critical because stock assessments can be improved with better estimates of spawning timing and duration, the age and size of the spawning population, sexual maturity, and fecundity. In addition, management can be further complicated if the sexual pattern of the species is protogynous hermaphroditism (e.g., changes sex from female to male) versus strictly gonochoristic (i.e., only has separate sexes) [2,33,34]. Based on sampling in the GOM in the 1970s and 1980s, primarily off the west coast of Florida, Bullock et al. [8] determined Goliath Grouper to be gonochoristic. This observation differed from their expectation of the species being protogynous, based on an earlier statement by Smith [15] that “most, if not all groupers are protogynous hermaphrodites”. More recently, Freitas et al. [35] sampled Goliath Grouper from Eastern Brazil and also found no evidence of hermaphroditism. However, weak indirect evidence of potential hermaphroditism has been observed in Goliath Grouper in the GOM [28] and in Bermuda [36], where presumably remnant fe-

male gonadal tissue (pre-vitellogenic oocytes) has been observed in mature testes. The reproductive pattern of Goliath Grouper in the GOM therefore remains unresolved. To date, the reproductive pattern of Goliath Grouper from the Atlantic coast of Florida is unknown, although they represent a significant spawning fraction of the population in the USA [10,23]. Protogynous hermaphrodites may be more vulnerable to overfishing than gonochorists if there is sex-specific fishing mortality [2,34,37]. Such selectivity can have severe adverse effects on monandric protogynous fishes (i.e., males arise only through sexual transition from mature females), whereby, in a size-selective fishery, males may be disproportionately targeted because they are more commonly the largest and oldest fish in the population. In monandric protogynous groupers, such as Gag *Mycteroperca microlepis*, this can lead to a marked reduction in males on spawning grounds and potential sperm limitation [2,38]. Protogynous hermaphrodites can also be diandric, however, whereby males arise either directly as primary males or through transitioning from mature females (i.e., secondary males) [39]. Diandric protogynous hermaphrodites are thought to be more resilient to over-exploitation because of the two pathways to becoming a male [39].

Life history data are essential to assessing the level of recovery in protected fish populations and in estimating population parameters, such as age distributions, growth, age-specific reproductive potential, and mortality rates [40]. These data are used as inputs into stock assessment models to predict recovery trajectories [26]. Although the overall USA Goliath Grouper population has been trending towards recovery since the 1990 harvest ban [22], the current lack of life history data makes it difficult to determine the level of recovery. Prior to the harvest ban, Goliath Grouper were aged using otoliths removed from harvested fish (i.e., lethal). Using this method, Bullock et al. [8] have provided the only known age and growth model for Goliath Grouper over a range of ages (0–37 years) and sizes (75–2160 mm TL) in the GOM, which remains the primary source of data used to inform the most recent composite growth model in the Goliath Grouper stock assessment [24]. Since the harvest ban and with the continuing lack of life history data for Goliath Grouper, it has been necessary to assume that the reproduction and growth of Goliath Grouper on the west coast of Florida adequately represents the reproduction and growth of fish from the east coast of Florida. This assumption has not held for other fish species in their patterns of growth between the US Atlantic and GOM stocks (e.g., Black Sea Bass *Centropristis striata*) [41].

For this study, our goal was to use non-lethal methods to directly determine the age, growth, and reproductive parameters of Goliath Grouper from the Atlantic (east) coast of Florida. Specific objectives were to (1) determine the sex-specific size and age structure of Goliath Grouper during their spawning season; (2) estimate sex-specific growth patterns; (3) determine the timing and duration of reproductive developmental stages and phases throughout the spawning season; and (4) determine the reproductive pattern (i.e., gonochoristic versus hermaphroditic) of Goliath Grouper from the Atlantic coast of Florida.

2. Materials and Methods

2.1. Fish Sampling

Goliath Grouper were captured by hook-and-line fishing off the southeast coast of Florida, USA, primarily offshore of Jupiter, at known aggregation sites (Figure 1) during the presumed spawning season of females (June–October) [8] in 2010–2016. Fish were captured using hand lines of braided nylon rope (9 mm, 60 m long) with monofilament leaders (1000 lb test, 5 m long) and baited 20/0 circle hooks. To lessen the effects of barotrauma, fish were captured in water depths <35 m and vented posterior to the pectoral fin when necessary. Goliath Grouper are not known to suffer immediate or delayed catch-and-release mortality due to barotrauma if vented appropriately [42]. Once caught, the fish was placed on a stretcher on the deck of the vessel, with a seawater hose inserted into its buccal cavity via the mouth to continuously irrigate the gills. A damp towel was draped over the eyes to protect them from direct sunlight and desiccation and to reduce visual stimuli.

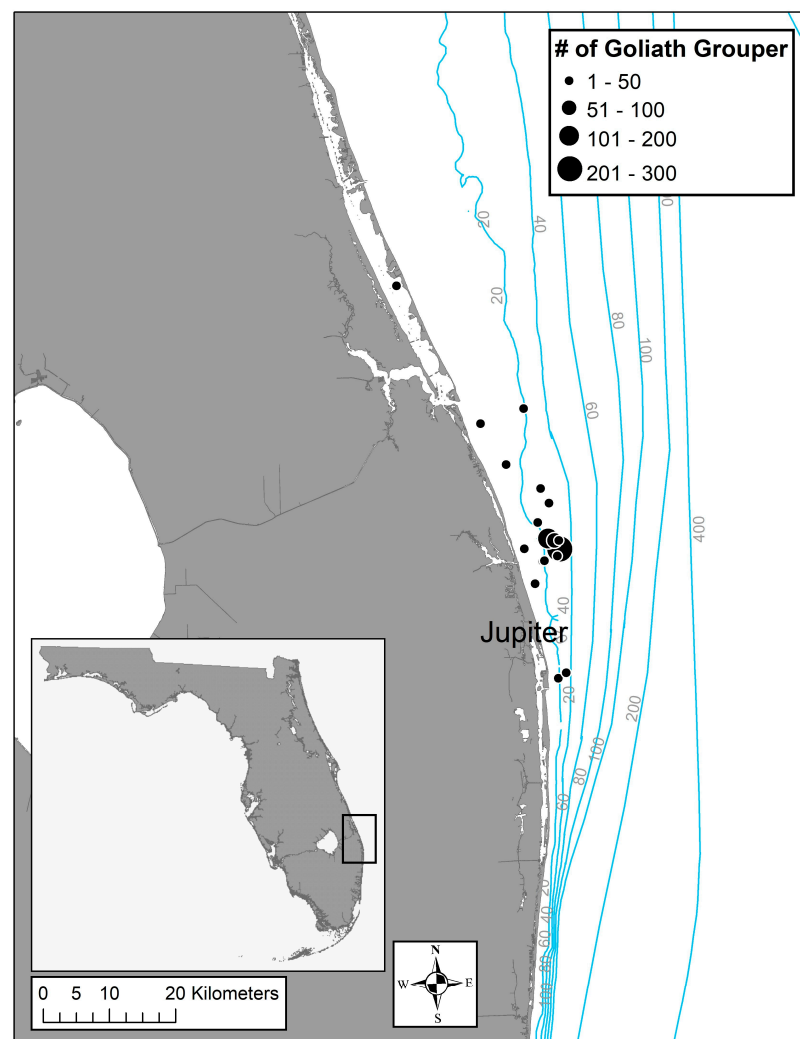


Figure 1. Sampling locations of Goliath Grouper from the Atlantic (east) coast of Florida (inset) around Jupiter during 2010–2016. Size of bubble indicates the number of Goliath Grouper sampled at each site. Bathymetric isobaths are in meters.

All fish captured were measured for total length (TL) to the nearest cm (from protruding lower jaw to end of tail). TL measurements from the top jaw (TJTL) were converted into lower jaw (LJTL) measurements using $LJTL = 1.0057(TJTL) + 1.3715$ ($n = 36$, $r^2 = 0.996$, size range of 100 to 189 cm TL). This conversion regression was based on paired UJTL and LJTL measurements taken from video recordings of 36 individual Goliath Grouper of various sizes, where a laser-based 20-cm scale was projected onto the fish by divers [43]. Captured still images of individual fish were measured for LJTL and UJTL using Q-image[®] digital calipers calibrated to the 20-cm laser scale. Length distributions of female and male fish were then compared using a Kolmogorov–Smirnov (K-S) test to determine if they were similar.

Before release, fish were also tagged externally with numbered cattle (PIG) ear tags and internally with passive integrated transponder (PIT) tags, if not previously tagged, to follow recaptures of individual fish over time. Each fish was sampled for ageing structures and their gonads were biopsied (details below). Some fish were not biopsied at all, however, either due to obvious sperm or egg release on capture, physical inability to remove any gonad material during the biopsy, or sampling time limitations.

2.2. Reproduction

All Goliath Grouper captured were externally sexed, when possible, based on the expression of milt or eggs upon capture, or by using gonoduct/vent properties [43].

Smaller fish, approximately <120 cm TL, were particularly difficult to sex externally using gonopores, and these fish, along with any other fish that had questionable external sex-identifying characteristics, were denoted as being of unknown sex for all sex-specific analyses. Due to the limitations of biopsies in small fish, size- or age-specific sexual maturity was not determined in the study.

Gonad biopsies were taken to further validate the sex of individual fish and to determine the stage of gonadal development. Females were biopsied by inserting a polyethylene catheter (6.3 mm OD, 4 mm ID) through the oviduct into the lumen of the ovary. The catheter was gently moved back and forth to remove ovarian tissue using a hand-operated vacuum pump (Mityvac MV8000) (Lincoln Industrial Corporation, St. Louis, MO, USA) with the tissue drawn into an in-line collection cup. Males were particularly difficult to biopsy due to the smaller diameter of the sperm duct, and so a smaller-diameter catheter (2 mm OD) was used to obtain these biopsies whenever possible. Some smaller fish could not be biopsied due to the physical constraints of the method. Extracted gonad tissue was fixed immediately in the field in 10% buffered formalin. Following preservation, the samples were transferred to 70% ethanol for storage until sent for histological processing at Crowder Histology Consulting (Baton Rouge, LA, USA). For histology, tissues were processed using standard paraffin embedding, cross-sectioned at 5–6 μm thickness, followed by staining with hematoxylin and eosin.

To assign gender using histology samples, the presence of female or male gonadal tissue, or a mix of both (hermaphrodite), was noted initially. For females, the presence/absence of all oocyte types was determined, which included oogonia, primary growth (PG), cortical alveolar (CA), early to late vitellogenic (VTG1, VTG2, VTG3), germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), hydrated (H), atresia (α - and β -), and post-ovulatory follicles (POF) (early and late) [44–46]. For males, cell types included spermatogonia (SG), primary and secondary spermatocytes (SC), spermatids (ST), and spermatozoa (SZ) [46].

To determine the reproductive cycles of mature females and males, the most advanced group of oocytes or the most advanced spermatogenic tissue, respectively, was identified and the percentage of each was plotted by month. Reproductive phases for females and males were assigned according to Brown-Peterson et al. [46], with modifications for biopsy-only samples. Female phases included immature (never spawned), developing, spawning capable (with the subphase actively spawning), regressing, and regenerating. Females with only PG oocytes were designated as immature if there were no signs of previous spawning (i.e., absence of atresia of vitellogenic oocytes, muscle bundles, POFs). Females with only PG and CA oocytes, and no signs of previous spawning, were considered to be in the developing phase but were designated as immature since they would not be expected to spawn in the current spawning season (i.e., they were sampled during the spawning season and had no vitellogenic oocytes) (as per [46]). Fish with VTG1 and VTG2 oocytes were in the developing phase and considered to be mature because it was expected that they would be able to participate in the current spawning season. Females with VTG3 or more advanced oocytes were considered to be spawning capable for the current spawning season. Females in this category that had GVM, GVBD, and/or hydrated oocytes were close to ovulation or spawning and were assigned to the subphase of actively spawning. Females with mass atresia and older POFs were considered to be regressing, although some vitellogenic oocytes may still have been present in these fish. Females with a prevalence of PG oocytes and signs of previous spawning (i.e., muscle bundles, thick ovarian wall) were considered to be in the regenerating phase; however, most biopsies did not sample the gonadal wall. Males were considered immature if only SG were present and in the developing phase if SG, SC, and ST were present in the absence of any SZ. All males with SZ were considered to be mature and spawning capable. In many cases, this designation was extended to the actively spawning subphase [46] due to milt being expressed when males were brought to the surface. The percentages of the reproductive phases among

mature females and males were plotted by month to determine the spawning season timing and duration.

The duration of the spawning season for females was determined by the difference between the first and last day that females were observed to be in the spawning capable phase, as well as in the actively spawning subphase. The spawning duration of males was determined similarly by using the spawning capable reproductive phase as the criterion.

Since many groupers are known to be protogynous hermaphrodites, all fish with female oocytes in the histological sections were thoroughly scanned for the presence of any male gonadal tissue that would indicate a female transitioning into a male. Similarly, all fish with male tissue were scanned for the presence of any remnant female gonadal tissue, which could indicate its transition from a female fish. Since the fish were biopsied and the gonads were not sampled whole, histological slides that showed male and female gonadal tissue that could have arisen through contamination during the catheterization or through the histological processing were not designated as transitional fish. For example, the presence of a loose VTG2 oocyte mixed in with spermatozoa (i.e., not embedded) could possibly have been due to contamination during the histological processing (e.g., floating oocytes not cleared from the water bath between samples). Therefore, to be conservative, fish were considered to be hermaphrodites only if female and male gonadal tissue was clearly observed to be physically embedded together.

The proportion of females to males relative to a 1:1 ratio was determined among 6 years of sampling (2010 to 2015) using a Chi-square goodness-of-fit analysis; the sample size was too low in 2016 ($n = 10$) to include it in the analysis.

2.3. Ageing Goliath Grouper Using Dorsal Fin Rays

Dorsal fin rays (hereafter referred to as “rays”) were non-lethally excised from all fish during their first capture. A subset of recaptured fish were resampled for rays to validate their use in ageing. Soft rays (3 and 4) of the second dorsal fin were removed by cutting across their bases as close to the pterygiophores as possible. In recaptured fish, the rays immediately adjacent to the previously excised rays were removed (i.e., not any regrown rays). Rays were prepared for ageing following the protocol outlined in Murie et al. [27], including cleaning each ray of extraneous tissue, air drying them, epoxying them in thermoplastic resin (Hysol[®], Loctite Corporation, Bay Point, CA, USA), and sectioning them using a variable high-speed sectioning saw (675 rpm) with a 152.4-mm-diameter blade. Rays were cross-sectioned in a range of thicknesses (0.8–1.3 mm), beginning at the base and continuing distally until the first annulus was observed merging with the core. This method provided multiple sections that were used to clarify the position of the first annulus, as well as the number of annuli compacted at the edge of the ray [27]. Sections were mounted on glass slides using Flotexx[®] (Lerner Laboratories, Pittsburgh, PA, USA) and aged using a zoom stereomicroscope (20–100 \times) and a compound microscope (400 \times) when necessary to view compacted annuli on the edge.

Rays were aged without knowledge of fish size or date of capture. Age class assignment was based on counting the number of translucent zones of the annuli, as well as the amount of opaque material at the edge of the ray relative to the previously completed annulus, given that an annulus comprises a translucent and an opaque zone combined ([27,47], this issue). Margin codes were assigned as follows: 1 = translucent zone on the edge, 2 = opaque material < 1/3 of the previously complete annulus, 3 = opaque material on edge between > 1/3 but less than 2/3, and 4 = opaque material on the edge > 2/3 of the previously completed annulus [48]. If the margin code was 1 or 2 between the capture dates of 1 January to 30 September, or if the margin code was 3 or 4 between the capture dates of 1 October to 31 December, then the age class was the same as the count of the translucent zones (e.g., referred to as annuli in rays). Fish captured from 1 January to 30 September that had an edge code of 3 or 4 had their age advanced one year (i.e., age class was their annulus count plus 1 year), and fish captured between 1 October and 31 December that had an edge code of 1 or 2 had their age demoted one year (i.e., age class was their annulus

count minus 1 year) ([47], this issue). This ensured that cohorts (year classes) could be tracked together through time [48].

Each ray sample was aged independently twice by the same reader (DJM) > 4 weeks apart. If both ages agreed, then the fish's age class was considered to be final. If the two ages did not agree, then the rays were read a third time. If two of the three ages agreed, then the age was final. However, if all ages were different, then the ray was re-aged and considered resolved when any two readings agreed. If all ages differed but at least three were all within 1 year of each other, then the median of these three ages was chosen as the final resolved age. If these conditions were not met after four readings, then the rays were considered unreadable and excluded from the database. Rays that were clearly cut too far above their base, and therefore did not capture the first few annuli of the ray, were also deemed unreadable.

The ray ageing method for Goliath Grouper was directly validated by sampling recaptured fish that had been at large for at least 1 year. Their age at recapture based on examination of their rays was then compared to their age at first capture plus the number of years that they had been at large between captures. The differences between these two ages were evaluated using a paired *t*-test. In addition, paired otoliths and rays sampled by Carroll et al. ([47], this issue) were aged independently by the primary reader of rays in this study (DJM) to determine the overall accuracy of the ageing used in this study. Within-reader precision was also estimated using the average percent error (APE) [49] to assess the consistency in the ageing method. Age distributions of male and female fish were compared using a K-S test to determine if they differed.

Year classes of Goliath Grouper were estimated by subtracting a fish's age class from its year of capture [48]. This was done for all unique fish to estimate any lag period between when the harvest of Goliath Grouper was closed in 1990 compared to the birth year of fish in this study.

Each fish was also assigned a biological (fractional) age to be used in the growth model analysis. The biological age was based on the difference between the peak spawning date (i.e., birth date) and their capture date as [48]

$$\text{Biological age (years)} = \text{Age Class (years)} + \left[\frac{\text{Capture Date} - \text{Birth Date}}{365} \right] \quad (1)$$

2.4. Growth

The growth of Goliath Grouper was analyzed by fitting the total lengths of individuals as a function of their biological ages to three commonly used growth models, including the von Bertalanffy, the Gompertz, and the logistic models [50]. Only unique individuals were used for the growth modeling (i.e., no repeated measures due to recaptured fish).

Growth models were fitted using nonlinear least-squares regression using the R-package FSA: Simple Fisheries Stock Assessment Methods version 0.9.4 [51].

The form of the von Bertalanffy growth curve was

$$L_t = L_\infty \left(1 - e^{-k(t-t_0)} \right) \quad (2)$$

where L_t was the predicted TL (cm) at time t (age, in years), L_∞ was the estimate of the average maximum length (asymptotic length) (cm), k was the growth coefficient (the rate of approach to L_∞), and t_0 was the theoretical age (years) when the fish length would be 0.

The Gompertz growth curve was

$$L_t = L_\infty e^{-e^{-G(t-t_0)}} \quad (3)$$

where G was the instantaneous rate of growth at age t_0 , and t_0 was the time at inflection of the curve at which the absolute age started to decline.

The logistic growth curve was

$$L_t = L_\infty / \left[1 + e^{-G(t-t_0)} \right] \quad (4)$$

Each model was checked for normality and homoscedasticity using residual plots provided in FSA [51]. Parameter estimates with bootstrapped 95% confidence limits (1000 runs) were calculated using the nlstools package in R [52]. Growth models were compared using Akaike's Information Criteria (AIC) [53] corrected for the small sample size (AICc) using the AICcmodavg package in R and Mazerolle [54]. The model with the lowest AICc was considered to be the most appropriate model; if differences between the model with the lowest AICc compared to the other two models (i.e., ΔAICc) were ≤ 2 , then the model with the least parameters was considered to be the most parsimonious [53].

Based on the most appropriate growth model identified, sex-specific models were then compared to a pooled-sex model using Likelihood-Ratio Tests (LRT) to check for coincident curves [40] using the R package growthlrt in fishmethods [55]. Growth curves were compared between females and males only over a similar range of fish age [40] and pooled when not significantly different from one another.

Individual fish that were recaptured in multiple years and measured one to four times throughout the study were assigned biological ages based on their recapture dates relative to the dates of their capture and direct ageing using rays. The longitudinal growth of these recaptured individuals was overlaid on the pooled growth curve based on the individual lengths and ages of unique individuals to qualitatively compare the longitudinal growth of multiple Goliath Grouper with growth based on the overall population.

The overall growth curve from this study was compared qualitatively with the growth curve from Bullock et al. [8] and the composite growth curve from the most recent stock assessment report on Goliath Grouper [24]. This latter growth curve included all the data from Bullock et al. [8] for the GOM, Brusher and Schull [56], and O'Hop and Munyandorero [57].

3. Results

3.1. Fish Samples

In total, 768 Goliath Grouper were captured off the Atlantic coast of Florida (Figure 1) between 2010 and 2016. These captures represented 653 unique fish and 118 recaptures, as tracked through PIG and PIT tags, with one fish not measured for TL (Table S1). Out of 652 unique fish measured for TL, the smallest fish captured was 82 cm TL and the largest was 228 cm TL (Figure 2). Females ($n = 257$) ranged from 122 cm to 228 cm TL and males ($n = 264$) from 104 cm to 225 cm TL. Females designated as transitioning into males ($n = 7$, see below) ranged in size from 108 to 169 cm TL, and males designated as having transitioned from females (i.e., secondary males, $n = 25$) ranged in size from 124 to 209 cm TL. Unknown-sex fish ($n = 100$) ranged in size from 82 to 211 cm TL. The female size distribution was significantly different from the male size distribution (K-S test: $D = 0.216$, $p < 0.0001$), with more females occurring at larger sizes than males. The female to male sex ratio did not differ significantly from 1:1 among sample years ($\chi^2 = 4.388$, $df = 5$, $p = 0.495$) and averaged 0.96:1 (± 0.09 , ± 1 SE). Of the 118 recaptures, 80 individuals were recaptured once, 24 were recaptured twice, 11 three times, and 2 were recaptured four times.

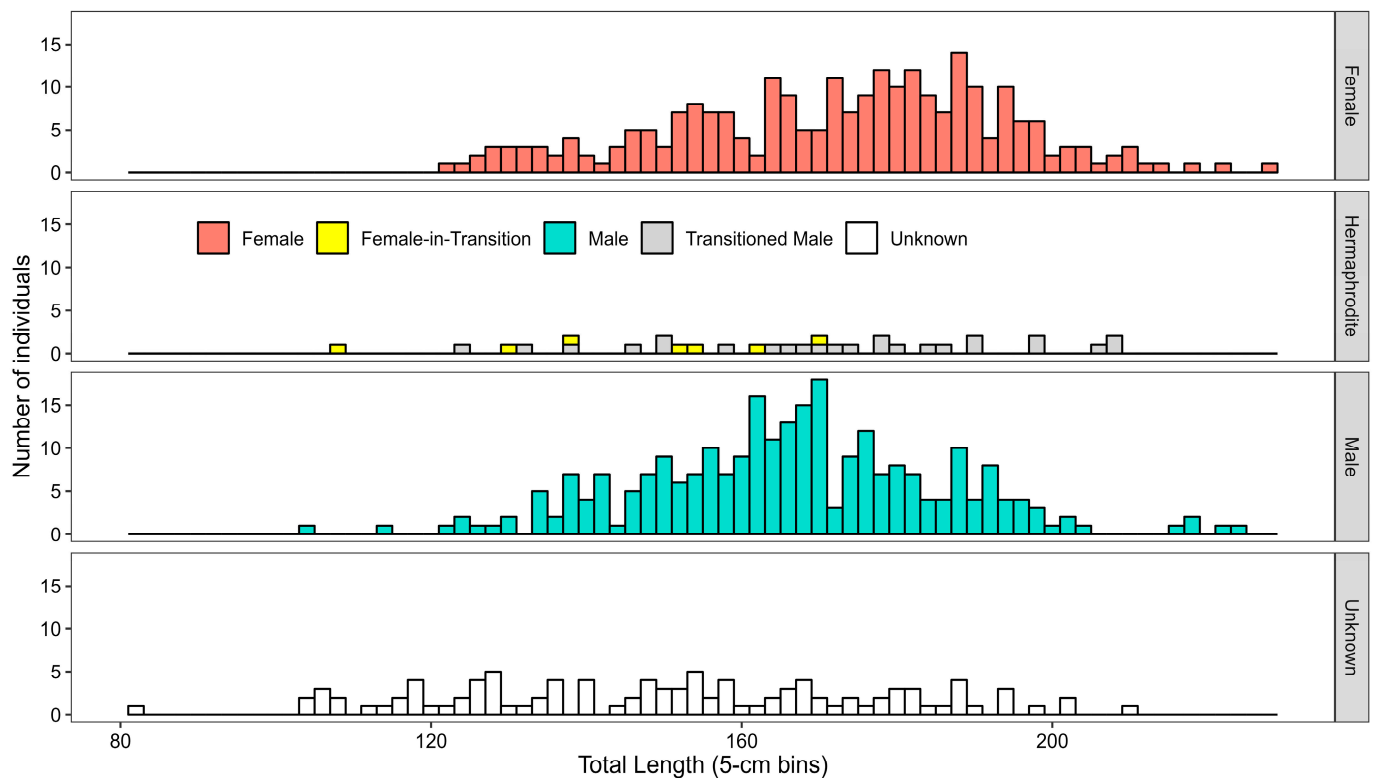


Figure 2. Length frequency distributions of Goliath Grouper sampled off the Atlantic coast of Florida for females ($n = 257$), hermaphrodites ($n = 32$), males ($n = 264$), and unknown-sex fish ($n = 100$). All hermaphrodites were sexed using gonad biopsies. Lengths are for individually unique fish (i.e., does not include recaptures) except for two females in transition and one transitioned male that were assigned an unknown sex at their first capture but were hermaphrodites on their second capture.

3.2. Reproduction

Females—Biopsies of female Goliath Grouper showed oocyte stages ranging from primary growth oocytes (PG) and cortical alveolar oocytes (CA) (Figure 3A) through vitellogenic oocytes (VTG1, VTG2, VTG3) (Figure 3B,C), as well as oocytes in final maturation going through germinal vesicle migration (GVM) (Figure 3D), germinal vesicle breakdown and yolk coalescence (GVBD/YC) (Figure 3E), and hydration (H) (Figure 3F). Biopsies also clearly showed early and late post-ovulatory follicles (Figure 3G,H, respectively), as well as oocytes going through both α -atresia (Figure 3I) and β -atresia.

A total of 259 females were staged for oocyte type (Figure 4A) and reproductive phase (Figure 4B) using biopsies. The frequency of the most advanced group of oocytes among the main sampling months of May through October indicated that the majority of females had primary growth (PG) oocytes in May, corresponding to most mature females being in a regenerating phase. These females had features of prior spawning, such as large blood vessels, large spaces around the PGs, and large connective tissue chords. Some females in May, however, also had cortical alveolar (CA) or early vitellogenic (VTG2) oocytes, which indicated that they were in a developing phase. A small percentage of females in May had late vitellogenic oocytes (VTG3), indicating that they were in a spawning capable phase. By June, most females were in vitellogenesis and in either developing or spawning capable phases. Females were actively spawning by July, as indicated by the presence of oocytes in GVM and hydration. In August through October, all or the vast majority of females were either in a spawning capable phase, as indicated by late vitellogenic oocytes (VTG3), or in the actively spawning subphase, as indicated by the presence of GVM, GVBD, and hydrated oocytes. Some females by October had a majority of atretic oocytes or primary growth oocytes as their most advanced group of oocytes, which indicated that they were in a regressing or regenerating phase, respectively, with their spawning completed. No

females were sampled in November, but the few fish sampled in December only showed primary growth oocytes and were in a regenerating phase.

Based on the first and last occurrence of late vitellogenic oocytes (VTG3) relating to the spawning capable reproductive phase, the maximum estimated duration of the spawning season for Goliath Grouper on the east coast of Florida based on all 6 years of sampling was from 26 May through 6 October, a total of 134 days. Within this timeframe, females were observed to be actively spawning, as indicated by oocytes in final maturation (i.e., hydration), from 16 July to 6 October, a total of 83 days.

Although age- and size-specific sexual maturity was not determined due to the difficulties in consistently obtaining biopsies from smaller and presumably younger fish, the smallest female biopsied that was mature was 123 cm TL and 7 years old, and the youngest female biopsied that was mature was 6 years old and 129 cm TL.

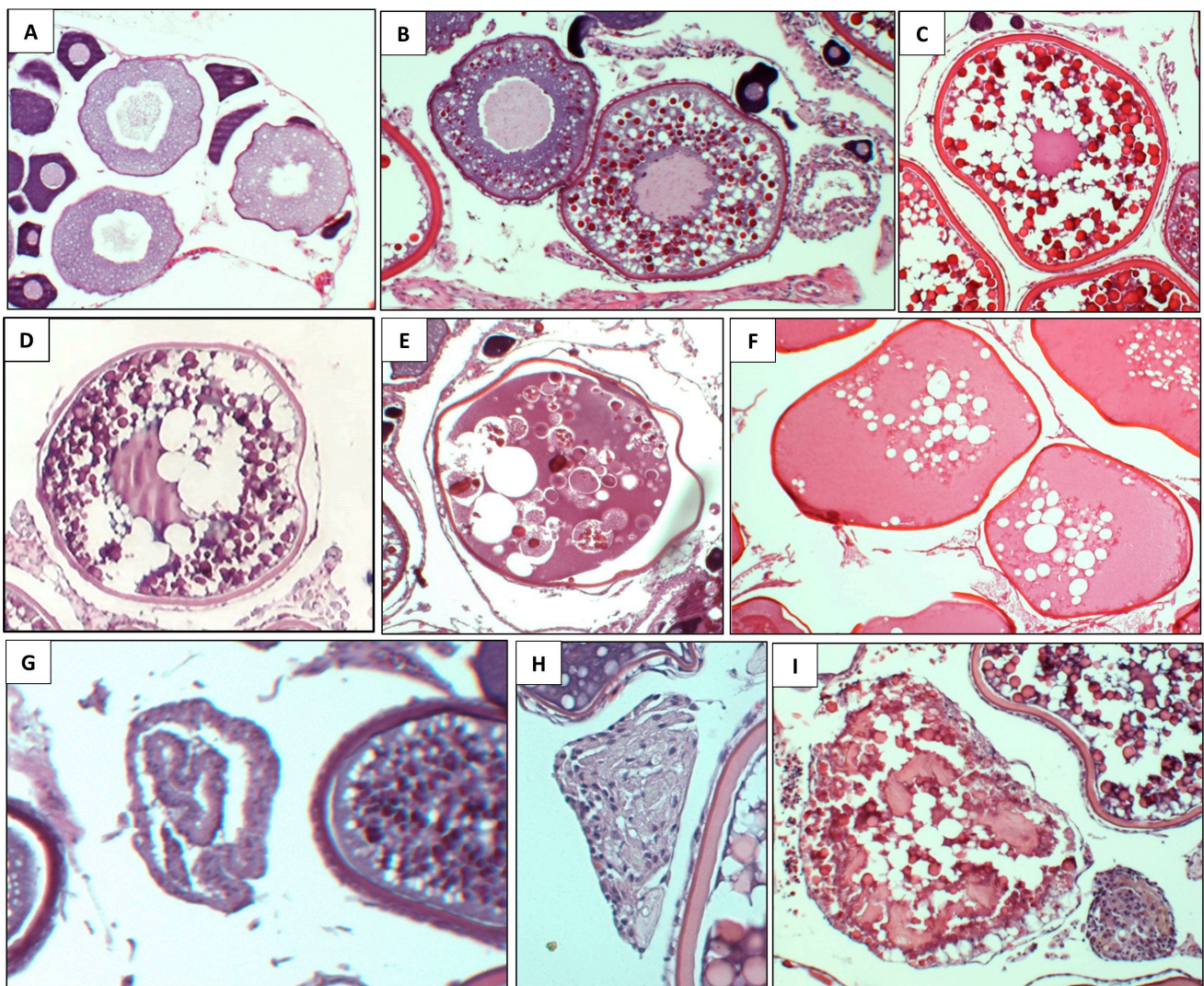


Figure 3. Oocyte stages identified from Goliath Grouper biopsies, including (A) primary growth (PG) and cortical alveolar (CA); (B) primary and secondary vitellogenic (VTG1 and VTG2, respectively); (C) tertiary vitellogenic (VTG3); (D) early germinal vesicle migration (GVM); (E) germinal vesicle breakdown (GVBD); (F) hydration (H); (G) early post-ovulatory follicle (e-POF); (H) late POF (l-POF); and (I) α -atresia.

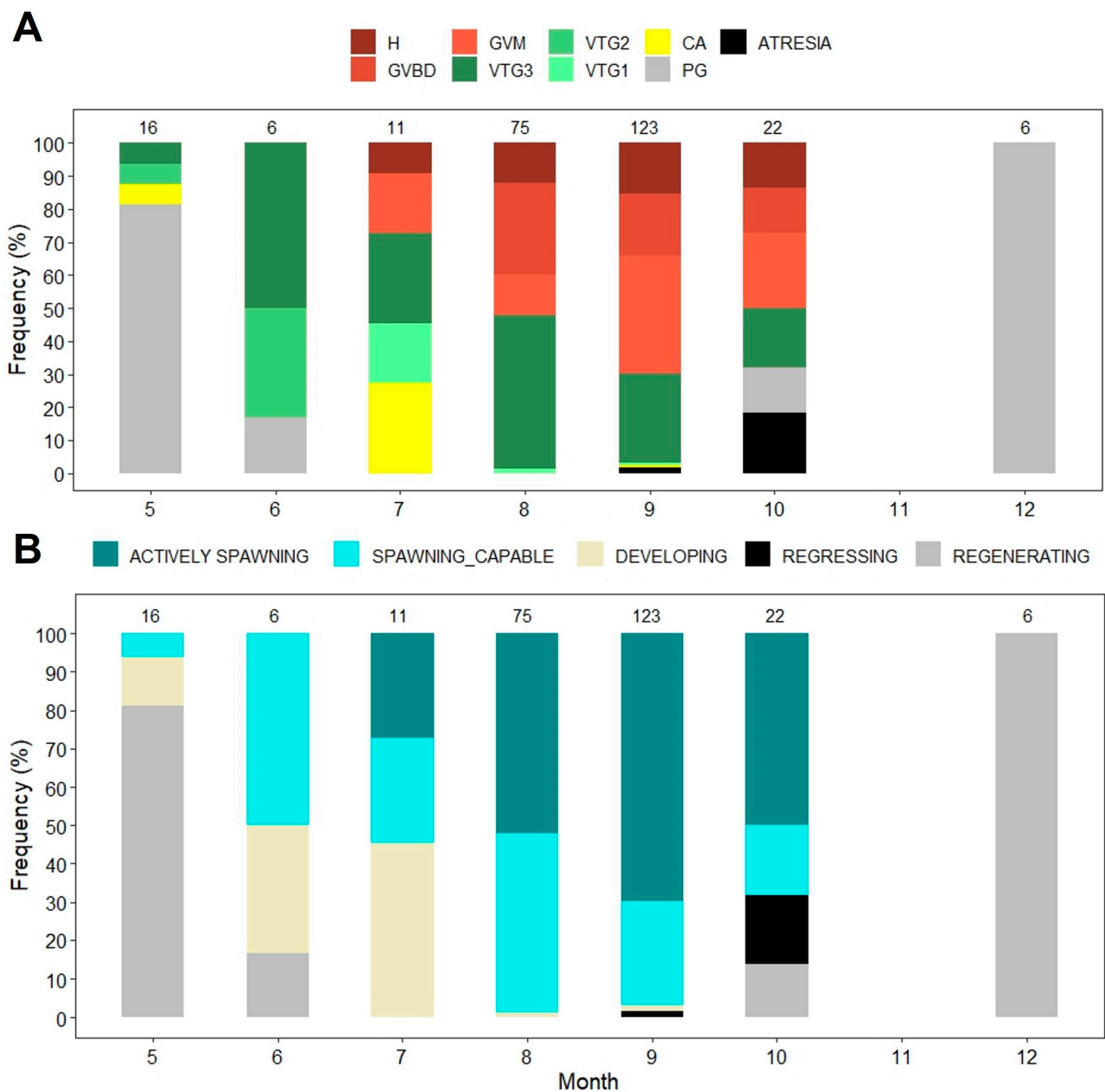


Figure 4. Percentage of mature Atlantic Goliath Grouper females with (A) various oocyte stages within each month and (B) reproductive phases each month. See Figure 3 for oocyte stages.

Males—A total of 124 males were staged using either biopsies or the expression of milt during capture. All stages of spermatogenic tissue were identified in males during the study period, including spermatogonia, primary and secondary spermatocytes, spermatids (ST), and spermatozoa (SZ) (Figure 5). All but five males were either spawning capable or in the actively spawning subphase throughout May to October, as determined by the presence of spermatozoa in the biopsies or the expression of milt during capture, respectively (Figure 6). Males in the spawning capable phase were most likely in the subphase of actively spawning based on the amount of milt in the histological samples but could not conclusively be placed in the actively spawning subphase if the expression of milt was not recorded during capture. For males, the youngest and smallest male sampled that was mature was 104 cm TL and 4 years old.

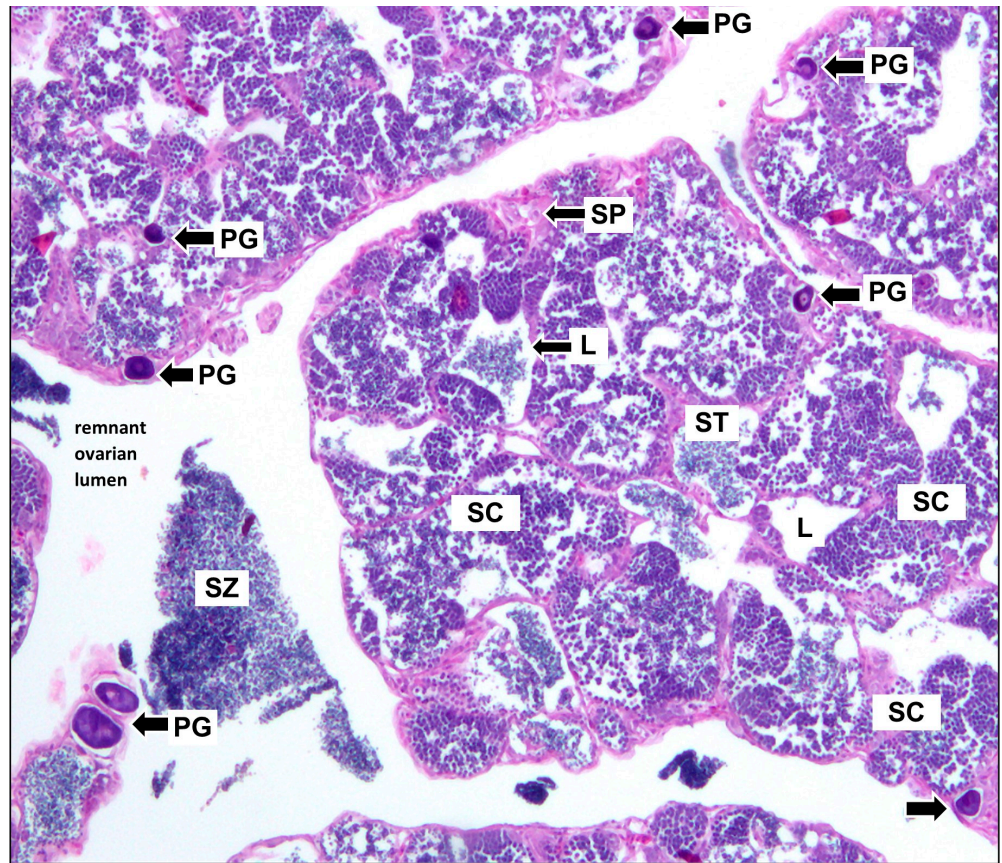


Figure 5. Histological sample from a biopsy from an actively spawning male Goliath Grouper showing all stages of spermatogenesis, including spermatogonia (SP), primary and secondary spermatocytes (SC), spermatids (ST), and spermatozoa (SZ); note the remnant primary growth oocytes (PG) throughout the tissue. The remnant lumen of the ovary is distinguished from the lumens of the lobules (L).

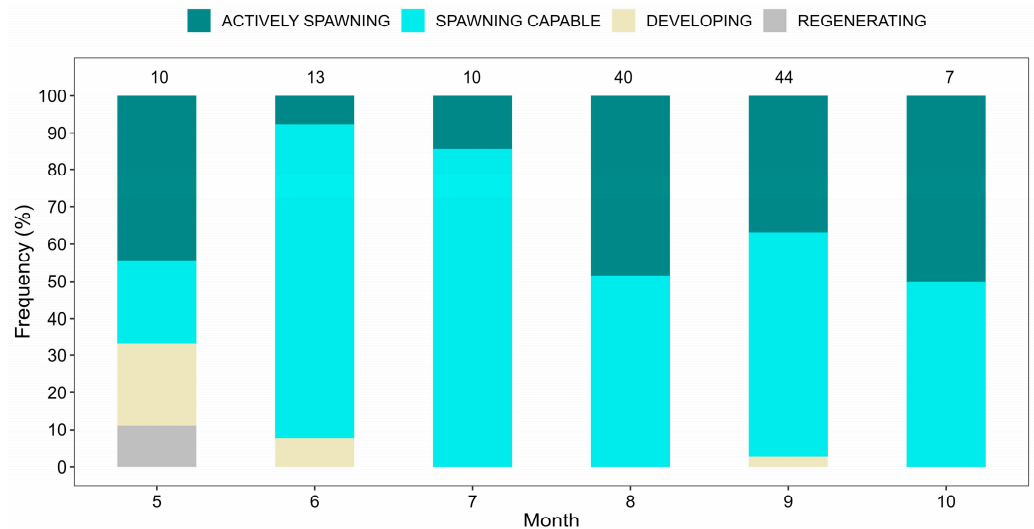


Figure 6. Percentage of Atlantic Goliath Grouper males in each reproductive phase by month.

Hermaphrodites—Hermaphroditic individuals ($n = 32$, or 4.9% of unique individuals) were also identified through gonad biopsies and recaptured individuals. In total, seven fish (1.1%) were identified as females transitioning into males (Figure 7). These individuals had a majority of female ovarian tissue that was variously infiltrated with male testicular tissue,

along with some atretic oocytes. Some of these females had only a few spermatogenic cysts embedded within their ovarian tissue, while others had both vitellogenic oocytes (VTG2 as the most advanced oocyte stage) and lumens of testicular lobules filled with spermatozoa (Figure 7). A total of 25 individuals (3.8%) were identified as males that had transitioned from females, with multiple primary growth oocytes embedded in testicular tissue (Figure 5). Remnants of ovarian lumen were also observed in many of these biopsied males (Figure 5). A further 10 males had potentially transitioned from females but did not meet the conservative criteria used to identify hermaphrodites in our study. These males only had either a single or a few PGs that were loosely associated with spermatozoa in the biopsy sample.

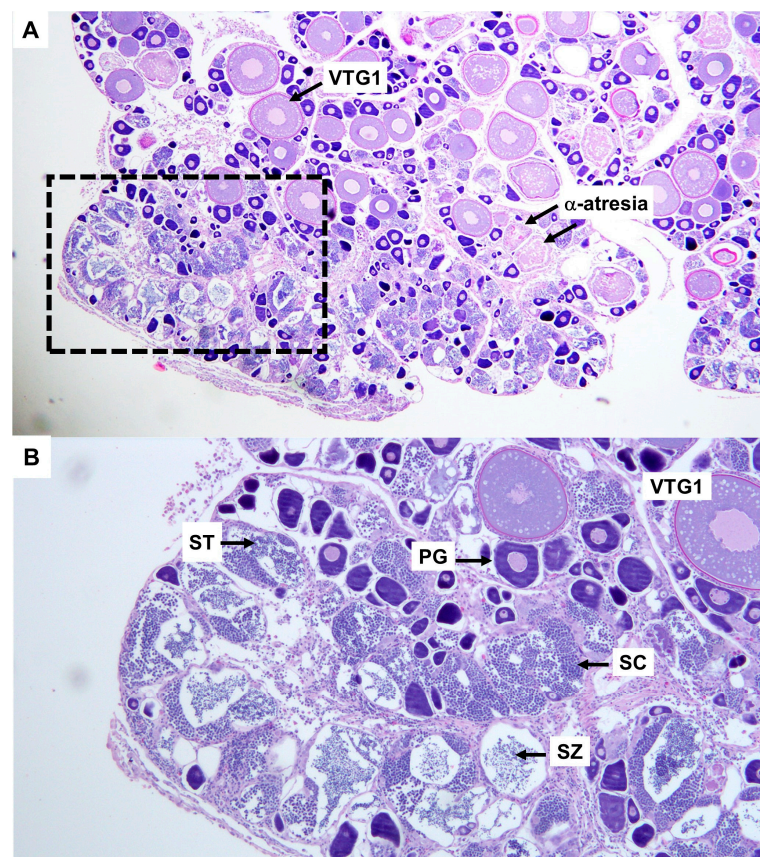


Figure 7. Female Goliath Grouper in transition to a male, showing (A) general organization of the female tissue relative to the embedded male tissue, and the dashed box enlarged in (B) to show the various female oocyte types (PG, early VTG1) and male features (SC, ST, SZ). See Figures 3 and 5 for female and male cell types, respectively. Note the presence of atretic oocytes.

In addition, the transition of two individuals from female to male was traced through recaptures and biopsies. One fish (Fish #80) initially identified via biopsy as a regenerating female in December 2010 was later recaptured and biopsied in June 2011 as a spawning capable male with spermatozoa. Another fish (Fish #200) was initially captured as an 8 year old in August 2011 and was identified as a female in transition to a male based on biopsy. This fish was identified as a male upon recapture by gonopores only in the following 2 years in September 2012 and August 2013. However, this fish was actively spawning (i.e., expressed milt upon recapture) in August 2015 as a 12 year old and its biopsy also showed spermatozoa but without any ovarian tissue present.

It was notable that a few of the transitional fish were relatively small and young but showed signs of prior spawning as a female, including a 104 cm TL 5 year old. Prior spawning markers in this transitional fish included oocytes in α - and β -atresia and macrophage centers (“brown bodies”).

3.3. Sex-Specific Age

Within-reader (DJM) average percent error (APE) for the precision in ageing Goliath Grouper from 3 to 22 years old using rays was 4.24%. In addition, the ray method of ageing Goliath Grouper was directly validated through the recapture of tagged individuals through time. In total, 32 fish had their ages determined directly from rays on their first capture, and subsequently a year or more later upon their recapture. Their age at recapture from directly ageing their rays was then compared to their age at first capture plus the number of years that they had been at large between their first and second capture. The mean difference between these ages was -0.33 years (± 0.28 years, ± 1 SE), for fish ranging in age between 8 and 16 years, and the paired ages were not significantly different from one another (paired t -test: $t = 1.09$, $p = 0.282$). In summary, 17 fish had perfect agreement between the two ages, 13 fish had a ± 1 year difference, and 2 fish had a ± 2 year difference.

Goliath Grouper ranged in age from 3 to 22 years of age (Figure 8), with 592 fish aged using rays. Captured females were 5 to 20 years of age and males were 4 to 22 years old. Females that were transitioning into males were between 5 and 10 years of age, while males that had transitioned from females were between 7 and 20 years of age. Fish of unknown sex were between 3 and 15 years of age. The cumulative age distributions of females and males were not different from one another (K-S test: $D = 0.119$, $p = 0.120$).

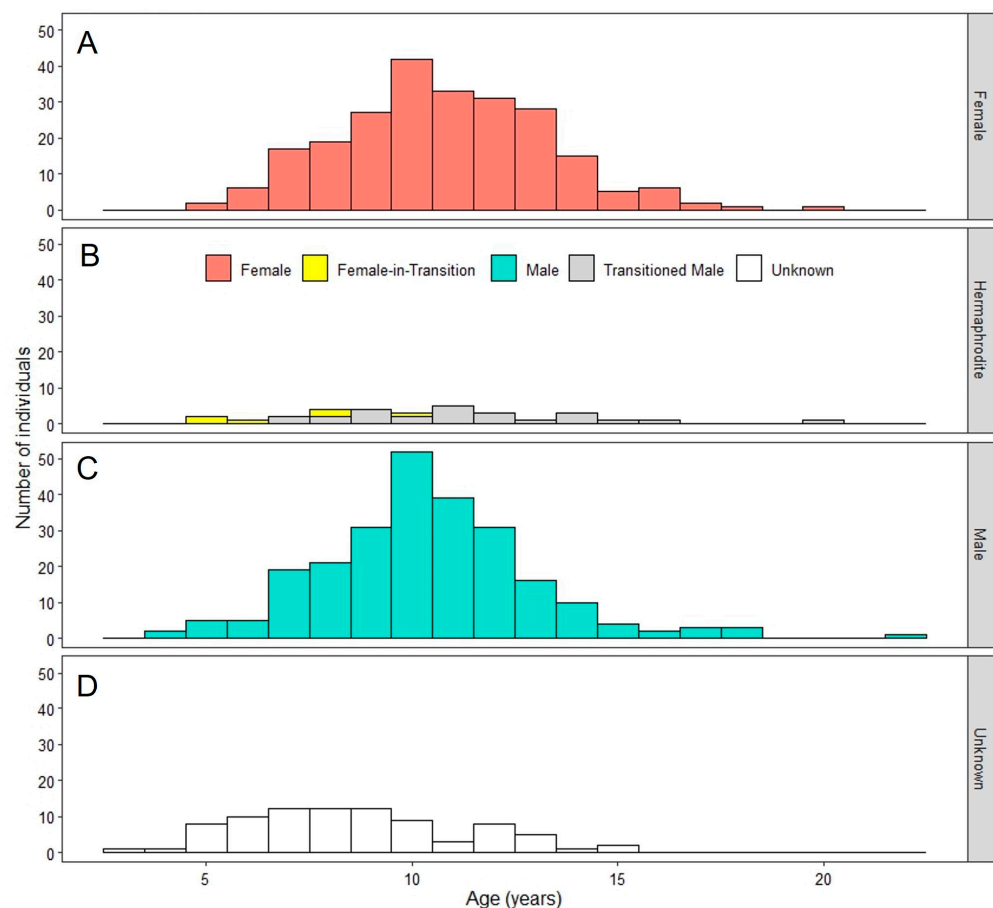


Figure 8. Age frequency distributions of Goliath Grouper sampled off the east coast of Florida: (A) females ($n = 237$); (B) hermaphrodites ($n = 29$); (C) males ($n = 244$); and (D) unknown-sex fish ($n = 82$).

Most Goliath Grouper sampled from the east coast spawning aggregations were born in 2002 and 2003 (Figure 9). Few fish were born between the closure in 1990 and 1995, with year classes after 1998 substantially higher. This indicated that there was an approximately 8-year lag between the closure and fish being born that survived to later enter the spawning

aggregations. The descending limb of the year class frequency indicated that for 2005 and later, either less fish were born and survived overall or fish that survived were not yet recruited to the spawning aggregations (i.e., not available to our sampling).

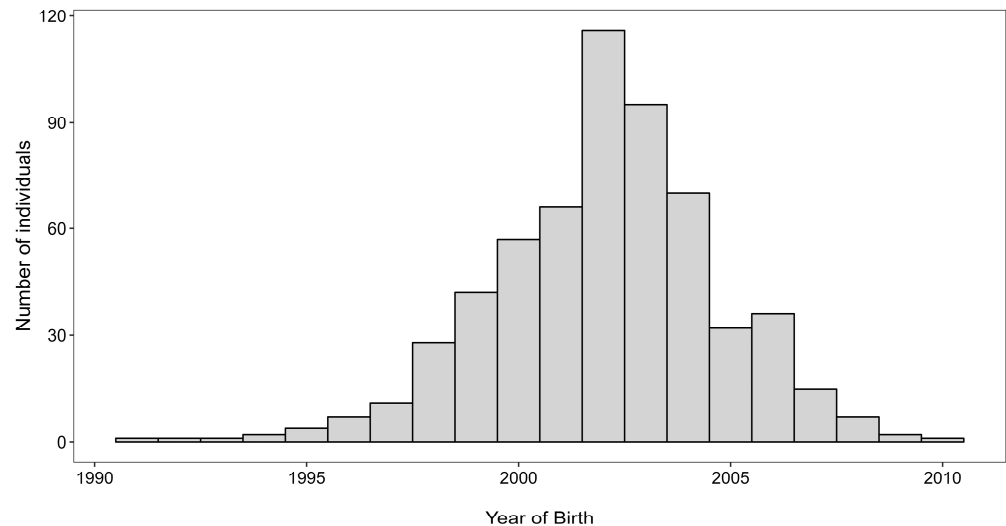


Figure 9. Year classes (birth years) of Goliath Grouper sampled off the east coast of Florida ($n = 592$).

3.4. Growth

The growth of Goliath Grouper from the Atlantic coast of Florida was modeled using the biological age based on a 1 September birthdate (see Section 3.2). Growth was best fit using a von Bertalanffy growth model, with a ΔAICc for the Gompertz and logistic models > 2 relative to the von Bertalanffy model (ΔAICc of 15.49 and 34.36 for the Gompertz and logistic model, respectively) (Table 1). Sex-specific growth between males and females was evident in Goliath Grouper, with the model including sex-specific parameters for L_{∞} , k , and t_0 (hypothesis H_4) explaining significantly more of the variation than the pooled-sex model where female and male parameters were combined (hypothesis H_0) (Table 2). The non-linear von Bertalanffy growth model indicated that, in general, females had a greater L_{∞} compared to males, with a concomitant decrease in k (Table 3), resulting in females being larger at age than males starting around 10 years of age (Figure 10A). The sex-specific growth difference was relatively small even in older, larger fish (i.e., ~ 10 cm TL difference in fish ≥ 15 years of age) (Figure 10A).

Table 1. Akaike's Information Criteria (AIC) for three candidate growth models with sexes pooled. K is the number of model parameters, AICc is the corrected AIC based on the small sample size, ΔAICc is the relative difference between the model with the lowest AIC compared to the other candidate models, AICc weight is the proportion of the total predictive power of the set of candidate models, and log likelihoods were used to calculate the AIC scores.

Model	K	AICc	ΔAICc	AICc Weight	Log Likelihood
von Bertalanffy	4	4898.12	0.00	0.99	−2445.02
Gompertz	4	4913.61	15.49	0.01	−2452.77
Logistic	4	4932.48	34.36	0.00	−2462.21

A pooled growth model was also estimated because Goliath Grouper are generally not able to be distinguished as male or female externally (other than by gonopores) and to allow comparison with previous growth models (i.e., [8,24]). This model pooled all fish with known ages, including males, females, fish of unknown sex, and fish designated as hermaphroditic (Table 3, Figure 10B). While growth was variable, hermaphroditic Goliath Grouper had similar growth as fish designated as females and males and were distributed throughout the overall growth curve (Figure 10B). In addition, the longitudinal growth

of 75 individuals captured and released over a period of 1 to 4 years during the study (Figure 11) also supported the generalized growth curve.

Table 2. Likelihood ratio tests for von Bertalanffy growth parameters (L_{∞} , k , and t_0) for male (M) and female (F) Goliath Grouper from the Atlantic coast of Florida. H_0 is the general model with separate parameters for each sex; parameters held constant for both sexes are given under the hypothesis column. Significant p -values are indicated in bold.

Tests	Hypothesis	Chi-Square	df	p -Value
H_0 vs H_1	$L_{\infty M} = L_{\infty F}$	1.94	1	0.164
H_0 vs H_2	$k_M = k_F$	0.24	1	0.624
H_0 vs H_3	$t_{0M} = t_{0F}$	0.38	1	0.538
H_0 vs H_4	$L_{\infty M} = L_{\infty F}; k_M = k_F; t_{0M} = t_{0F}$	8.67	1	0.034

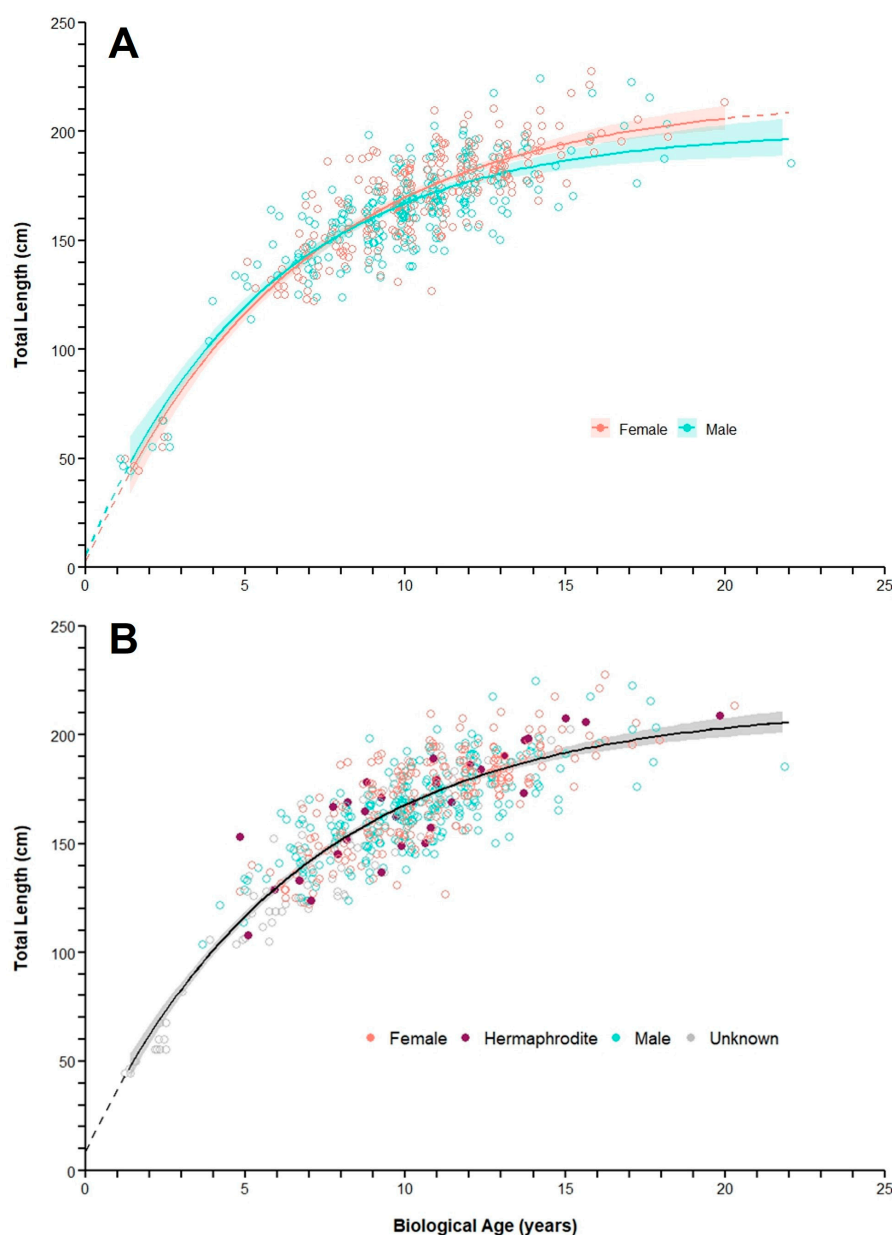


Figure 10. (A) Growth of female and male Goliath Grouper sampled off the Atlantic coast of Florida, USA, during June to October 2010–2016, and (B) growth of all fish sampled, regardless of sex. Solid lines are the predicted von Bertalanffy growth curves with 95% confidence limits shaded.

Table 3. Von Bertalanffy growth model parameter estimates (L_{∞} , k , and t_0) ($\pm 95\%$ confidence interval, CI) for Goliath Grouper from the Atlantic coast of Florida.

Sex	n	L_{∞} (cm)		k		t_0	
		Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Females	243	215.85	206.54–227.37	0.153	0.131–0.177	−0.089	−0.449–0.375
Males	251	200.32	190.33–214.82	0.177	0.138–0.214	−0.157	−0.989–0.295
All Fish	607	213.22	206.27–222.89	0.150	0.132–0.169	−0.283	−0.676–0.00

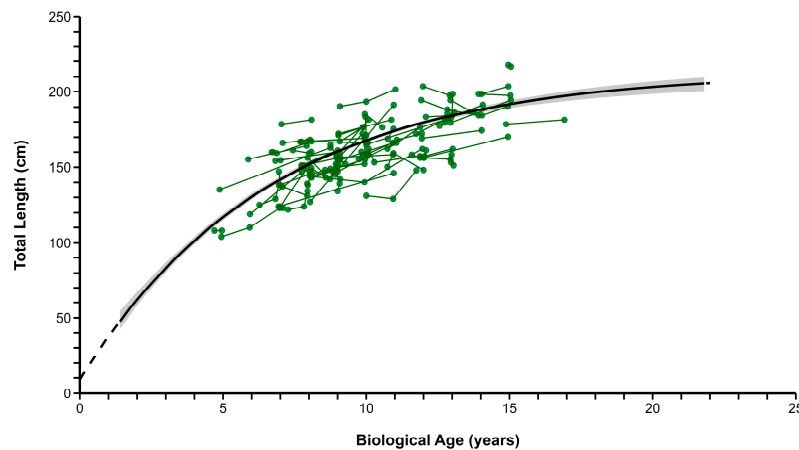


Figure 11. Growth of uniquely identified Goliath Grouper over multiple recaptures throughout the study period. Each series of green dots and connecting lines represents an individual, uniquely tagged fish. The black solid line is the overall predicted von Bertalanffy growth curve for all fish given in Figure 10B, with the 95% confidence limits indicated by gray shading.

Based on the comparative growth curves, Goliath Grouper from this study were larger at ages ~4 through 22 years compared to Goliath Grouper in the GOM and the overall composite growth curve (Figure 12). Whereas the growth model from Bullock et al. [8] paralleled the Atlantic coast growth curve for fish ≥ 10 years with a ~15 cm TL difference, the composite growth curve [24] intersected the Atlantic coast growth curve at around 25 years of age (i.e., beyond the maximum age of the grouper observed in our study). The composite growth curve also intersected the GOM growth curve at approximately 15 years.

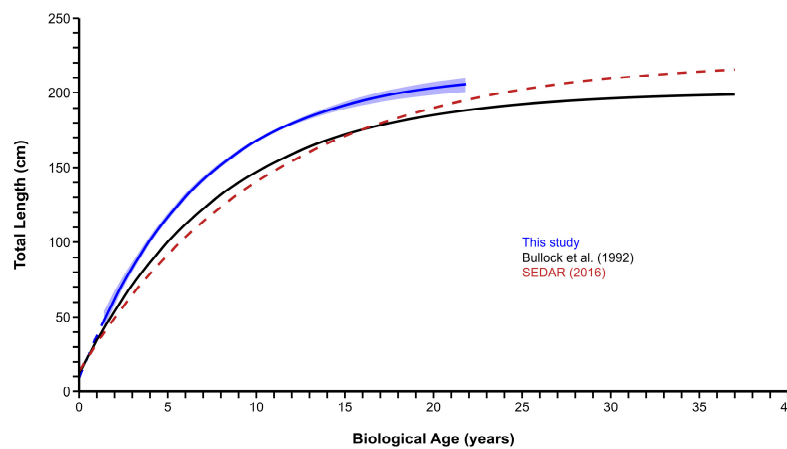


Figure 12. Von Bertalanffy growth curves for Goliath Grouper in the Southeastern USA, either sampled off the Atlantic coast of Florida (blue line, this study, with 95% confidence limits), in the Gulf of Mexico based on Bullock et al. [8] (black line), or from a composite growth curve used in the SEDAR [24] stock assessment based on Bullock et al. [8], Brusher and Schull [56], and O’Hop and Munyandorero [57] (red dashed line).

4. Discussion

For the first time, we have conclusively demonstrated that Goliath Grouper from the east coast of Florida are indeed protogynous hermaphrodites. Sadovy and Shapiro [58] state that the most direct and conclusive evidence for protogyny is found through gonad biopsies, in which individual fish are observed to first function as a mature female and then later as a mature male (i.e., functional sex change). The large number of Goliath Grouper tagged, recaptured, and biopsied during our study allowed for the direct observation of the transition from female to male in two individual fish during the time period of the study. Initial biopsies performed on these fish indicated that they were females but subsequent biopsies in the following years indicated that they were spawning as males (spermatozoa present in lumens of testicular lobules and also, in one fish, flowing milt upon recapture), thus providing conclusive evidence that Florida east coast Goliath Grouper have the ability to function as protogynous hermaphrodites.

It is relatively uncommon for individuals to be tracked in this manner over multiple years, however, and therefore only indirect evidence for protogynous hermaphroditism that relies on the observation of remnant female features in the testes of the male is available [58]. In relation to this, Atlantic coast Goliath Grouper appear to have an undelimited type 2 gonad whereby the female and male gonadal tissues are intermixed, rather than being separated by either a membrane (delimited) or by adjacent positions (undelimited type 1, separated but not by a membrane) [58]. For protogynous hermaphrodites, the features of ovarian tissue that are usually retained in the testes and are most easily visualized include the remnant ovarian lumen and the ovarian lamellar structure, as well as primary growth oocytes.

The central lumen observed in the testes can only be a remnant of an ovarian lumen if it is membrane-lined and does not transport sperm, and instead sperm sinuses in the gonadal wall are used for sperm transport [58]. The presumed ovarian lumen observed in the testes of transitioned males in our study was membrane-lined but there were clearly spermatozoa present in many of them. However, because biopsies reveal by default only a small portion of the gonadal tissue, it was possible that the spermatozoa present in the lumens of the testicular lobules were the sources of the spermatozoa in the remnant lumen. In addition, biopsies seldom sampled the gonadal wall and so the presence of sperm sinuses could not be used as indirect evidence of a transitioned male. The sampling and preservation of whole testes would allow a clear assessment of the presence or absence of spermatozoa in the presumed remnant of the ovarian lumen and the presence of sperm sinuses, but this would require lethal sampling and our sampling was intended to be entirely non-lethal.

The presence of primary growth oocytes in testicular tissue is not, on its own, decisive evidence of protogyny [58]. However, given the direct evidence of protogyny via the serial recapture of sexed fish and other indirect lines of evidence, the presence of embedded growth oocytes was also used to identify males that had most likely transitioned from females. This criterion was used conservatively and only in testes with primary growth oocytes that were clearly embedded in the testicular tissue. This was to negate any concerns that oocytes could be observed in testicular tissue due to contamination during the actual field biopsy or during histological processing. The proportion of male Goliath Grouper that had transitioned from females could be marginally higher than reported here if more liberal criteria were applied (i.e., 5.3% of unique fish). In addition, it is currently unknown whether ovarian tissue is completely resorbed in transitioned males and, if so, the timeline involved in this process. The one transitional fish that was recaptured as a mature male after 4 years had no ovarian tissue in its biopsy, but this alone does not provide a timeline because the biopsy method has limitations. In particular, although biopsies are representative of the gonad, only the collection of the entire gonad would allow for a full gonadal analysis to determine the presence of remnant female tissues. More males may have arisen from females through a functional sex change, therefore, but it was not possible to determine the extent based on biopsies alone.

Many epinephelid groupers have been characterized as being protogynous, primarily monandric [36,59]. Fewer have been designated as having a diandric protogynous

sexual pattern, including the Catface Grouper *E. andersoni* [59], Orange-Spotted Grouper *Epinephelus coioides* [60], and Giant Grouper *E. lanceolatus* [61] (a close relative of the Atlantic Goliath Grouper [62]). Other large epinephelids have been classified as functionally gonochoristic (e.g., Leopard Grouper *Mycteroperca rosacea*) [63], some with the potential to be protogynous (e.g., Nassau Grouper [64]; Camouflage Grouper *E. polyphkadion* (= *microdon*) [34]). Multiple lines of evidence suggest that the Goliath Grouper on Florida's east coast are diandric. First, the age and size distributions were not bimodal or skewed towards males being larger and older than females, which would typically be the case if all males were only derived by transitioning from smaller and younger females (i.e., monandry) [58,64–66]. This has been observed in monandric protogynous groupers including the Gag [38] and Brown-Marbled Grouper *E. fuscoguttatus* [65]. In contrast, groupers known to be diandric, such as the Catface Grouper, have largely overlapping size and age distributions between females and males [59]. Second, the sex ratio of female to male Goliath Grouper from the east coast was close to 1:1, which is not characteristic of a monandric protogynous grouper, where the sex ratio is typically skewed (highly skewed in exploited species) towards females; a 1:1 sex ratio is more characteristic of a gonochoristic species [58]. In the diandric Catface Grouper, the sex ratio was skewed towards males (1:1.34) [59]. Third, mature males were observed at smaller and younger ages than females in our study, with fish identified as mature males as small as 104 cm TL and 4 years old versus mature females as small as 123 cm TL and 6 years old. In diandric groupers, these small, young, mature males would presumably represent primary males that have arisen directly via sexual differentiation from the juvenile phase (i.e., not from females), in contrast to secondary males that arise through transitioning from females [59]. However, a diandric pathway cannot be determined conclusively without sampling smaller and younger fish to a greater extent. Since this sampling would need to be lethal (gonad biopsies are not currently successful in these smaller fish), it would be judicious to sample the gonads from the presently ongoing limited harvest of juvenile Goliath Grouper to histologically stage and sex the fish. Otherwise, a carefully planned research program selecting the exact locations and sizes of fish to be lethally sampled could also obtain the necessary information. These samples could also help to determine if Goliath Grouper go through an immature bisexual stage of development prior to sexual differentiation into either a female or a primary male, as observed in the Nassau Grouper [64] and Orange-Spotted Grouper [60]. This would further complicate the distinguishing of primary from secondary males because both males would show the same testicular morphology, including a residual ovarian lumen [58,67]. Understanding such potential plasticity in sexual differentiation in the juvenile stage is important because juvenile Goliath Grouper occur in different habitats from the adults and therefore the cues/triggers for juvenile sexual differentiation (i.e., social, environmental, settlement conditions, etc.) may be very different from the cues/triggers used by adult females to transition into secondary males.

It was perplexing that we observed only ~5% of the Goliath Grouper on the Atlantic coast of Florida showing any protogynous features. This may relate to the proportion of fish transitioning at any one time being very low, or that the transition and resorption of remnant ovarian tissue occurs relatively quickly (currently unknown). Alternatively, Goliath Grouper could rely on the primary male pathway more so than the secondary male pathway. If the majority of males are derived through the primary male route, then the length and age frequencies would overlap throughout their range and the sex ratio would be close to 1:1 [58], as observed in our study. Conversely, if the secondary male route was the most prevalent, then the bimodal size and age frequencies would be expected to follow that of a typical monandric grouper, with the proportion of males greater in the larger and older frequencies, and the sex ratio would be biased towards females [58], which was not observed in our study. It would be very difficult to distinguish the proportion of males derived from a primary versus secondary male pathway in Goliath Grouper because all groupers presumably start from ovarian tissue and then differentiate early on into either a female or male, and so all fish have an ovarian lumen and ovarian lamellar structure, includ-

ing both primary and secondary males [58,67]. In addition, both primary and secondary males form sperm sinuses in the gonadal wall for sperm transport, and hence this cannot be used to distinguish the two male pathways [64,67]. Given these limitations, the most parsimonious conclusion is that Goliath Grouper on the east coast of Florida have ~50% of their population as primary females (i.e., gonochoristic females) [68] and a substantial (but unknown) proportion of their population as primary males (i.e., gonochoristic males), with a more minor proportion as secondary males. Sadovy and Colin [64] considered that the sexual pattern of a species should be characterized by the extent of functional sex change (i.e., mature females changing into males) and considered Nassau Grouper to be essentially or “functionally” gonochoristic because “If. . . males develop predominantly from bisexual juveniles and rarely from sex-changed females, the sexual pattern is functionally gonochoristic” [64]. Conversely, “If. . . a substantial number of males derive through the sex change of adult females, the sexual pattern is best characterized as diandric” [64]. Under these considerations, the Goliath Grouper from the east coast of Florida appear to be functionally gonochoristic, with the potential for protogynous hermaphroditism, similar to the Nassau Grouper and supported by the sexual pattern observed by Bullock et al. [8] for GOM Goliath Grouper.

Although there have been some previous indicators that Goliath Grouper may be a protogynous hermaphrodite, the data have been indirect or inconclusive. Testes from one 1.8 m male Goliath Grouper collected in Bimini were reported as having remnants of many ova [36]. More recently, Collins and Barbieri [28] noted that three of nine males sampled from the GOM showed primary growth oocytes in their testes, as well as a lamellar structure and a presumed ovarian lumen. These observations offer indirect support for protogyny in GOM Goliath Grouper. In contrast, however, Bullock et al. [8] did not find any histological evidence of protogyny during their study. They collected females and males with substantially overlapping age distributions, as did Collins and Barbieri [28]. In addition, Bullock et al. [8] reported that males matured at slightly smaller sizes and younger ages than females. As a whole, these observations would not be expected if Goliath Grouper were monandric protogynous hermaphrodites but could provide indirect support for them being functionally gonochoristic with the potential for diandry in the GOM, similar to our suggested sexual pattern for Goliath Grouper from the east coast of Florida.

We suggest that the occurrence of hermaphroditism noted in this study, based solely on biopsied gonads, provides a conservative estimate of hermaphroditism in Florida Atlantic coast Goliath Grouper. Males caught during field studies that were brought to the surface spewing milt were not always biopsied, even though they might have had residual female tissue in their testes. Further, evidence of transition can be missed even in biopsied females if the catheter misses the testicular tissue infiltrating the ovaries. Therefore, hermaphroditism in Goliath Grouper may occur in greater frequency than determined through gonad biopsies alone, although Bullock et al. [8] sampled whole gonads histologically and found no instances of hermaphroditism.

In our study, transitional individuals were identified both as females in transition to males and as males that had transitioned from females. A few females with extensive male gonadal tissue could have been categorized as mature bisexuals—that is, mature female and male reproductive tissues in the same fish, as occurs in some Nassau Grouper [64]. As females in transition, these fish were mature but not spawning capable (i.e., no VTG3 or later oocytes). However, female Goliath Grouper in transition had atretic oocytes and α - and β -atresia, indicating that they had most likely spawned previously but, without directly observing POFs, which are short-lived in warm water reef fishes (resorbed within ~24–48 h [69,70], this could not be conclusively determined.

Overall, transitional individuals usually represent a low proportion of fish at any one time [58] (e.g., 1.25% of Gag [66]). Based on all of the individuals that could be measured and sexed in our study, ~1.1% sampled during the spawning season were in transition (7 out of 653 fish) and 3.8% had already transitioned. Triggers for transition from female to male are complex and may include a variety of social, size-related, and environmental cues [38,71].

The timing of sexual transition in groupers can vary, occurring within or at the end of the spawning season, or in the interval between spawning seasons [38,58,71]. We observed Goliath Grouper with mostly female gonadal tissue, with some testicular tissue present on the spawning grounds, primarily captured during August and September (i.e., during peak spawning season). These transitional fish spanned relatively large size (108–191 cm TL) and age (4–12 years) ranges. One of the Goliath Grouper females sampled near the peak of the spawning season in August was mature and had VTG2 oocytes, indicating that she would be expected to spawn in the current season. However, we also observed mature male gonadal tissue (including spermatozoa) in her gonad, which indicated that she was transitioning to a male. Transition from a mature female to a male with functioning testes can occur as quickly as 3–7 weeks in epinephelid groupers, based on the Rock Hind *Epinephelus adscensionis* (~50 days) [72] and the Half-Moon Grouper *E. rivulatus* (3 weeks) [71]. Goliath Grouper therefore appeared to be cued to transition within the spawning season. However, since our sampling was mostly restricted to spawning sites and the spawning season, it would be beneficial to sample fish outside of this time and area to observe whether fish also transition in the post- or pre-spawning periods.

Based on the first and last occurrence of oocytes in final maturation, the spawning season for Goliath Grouper on the Atlantic coast of Florida was ~83 days in duration. However, the periodicity of spawning activity has to be considered when estimating the spawning season duration. Goliath Grouper off the east coast of Florida exhibit peak spawning activity during the new moons of August, September, and October ([10, which includes fish from this current study]). Our sampling extended the active spawning season to include July, although it clearly occurred at a lower level. Since the majority of females spawn or have very recently spawned within ± 3 –4 days of the new moon [10], this suggests that active spawning is in fact limited to only 28 days (four new moons over the four spawning months, with spawning occurring for ~7 days on each new moon phase). While more refined estimates of the spawning season duration could be quantitatively modeled using circular statistics, this rough estimate serves to remind us that the spawning season for groupers, as well as other fish groups that spawn in synchrony with lunar phases, are relatively restricted in their egg production compared to species that spawn on a daily basis or multiple times per week throughout all months of their spawning season (e.g., Red Snapper *Lutjanus campechanus*, every 2–5 days [73]).

Bullock et al. [8] reported that female Goliath Grouper in the GOM mature at 120–135 cm TL and 6–7 years old and males mature at 110–115 cm TL and 4–6 years. The smallest size and youngest age of females and males sampled from the east coast of Florida that were mature were similar to the ages and sizes of maturity in the GOM, with the exception of a small (104 cm TL) female in transition that appeared to potentially have spawned previously. Bueno et al. [9] reported mature females that were 100 and 119 cm TL from Southern Brazil, and Freitas et al. [35] estimated the size at first maturity for females from Abrolhos Bank in Eastern Brazil at 106 cm TL. These slightly smaller sizes at first maturity in Goliath Grouper from Brazil may indicate regional or exploitation differences among these populations.

Goliath Grouper on the Atlantic coast of Florida sampled during 2010–2016 appeared to have a younger demographic than that encountered by Bullock et al. [8] in the GOM between 1977 and 1990, prior to the fishery closure. Most fish aged by Bullock et al. [8] were ≤ 18 years old (~78%), but ~22% were relatively old (> 18 years, up to a maximum age of 37 years). In contrast, nearly all of the Goliath Grouper sampled from the east coast of Florida from 2010 to 2016 were ≤ 18 years old (99% of aged fish). However, in a 20–26-year post-closure period, at the time of our study, the east coast population still appeared to be skewed towards relatively younger fish compared to fish in the GOM in 1990, the opposite of what would be expected in a recovering population. Indeed, these results suggest that the fishery closure did not result in an immediate increase in juvenile Goliath Grouper on the east coast of Florida that survived and joined the spawning aggregations sampled in our study, but rather incurred a lag period of at least 8–12 years.

Whereas the age distributions of females and males from the Atlantic coast of Florida were similar, the size distribution of females was slightly larger than that of males at age. This difference could be biologically significant, given that larger females are more fecund than smaller females [74,75]. This was also reflected in the sex-specific growth of Goliath Grouper from the Atlantic coast of Florida, with females being larger at age than males when >10 years old. This was unexpected, given that Bullock et al. [8] did not observe sexually dimorphic growth in Goliath Grouper in the GOM. However, ~70% of fish measured by Bullock et al. [8] were unsexed, and the small sample size of fish that were sexed, measured, and aged may have prevented a robust analysis of dimorphic growth.

The growth curve for Goliath Grouper from the Atlantic coast of Florida should be incorporated into future population or stock assessments since it consistently showed fish that were larger-at-age compared to the SEDAR [24] composite growth curve. The SEDAR [24] growth curve included data from Bullock et al. [8] from the GOM, as well as additional data for ~1100 juveniles (0–6 years old) from Brusher and Schull [56] and ~100 fish mostly <6 years old collected by the Florida Fish and Wildlife Research Institute from cold kills or red tides [57]. The inclusion of many juvenile fish, compared to relatively small numbers of very old fish, resulted in a growth model with a smaller k and a concomitantly greater L_{∞} (222 cm TL) compared to Bullock et al. [8], where L_{∞} theoretically represents the average maximum size of fish. However, the SEDAR [24] composite growth model appeared not to fit the data particularly well given that the L_{∞} of 222 cm exceeded the maximum size of any Goliath Grouper measured and aged by Bullock et al. [8] (216 cm TL), and, in addition, most of the length data for fish >25 years old fell below the modeled curve (i.e., the regression line did not represent the average maximum size for fish >25 years of age). This emphasized that the composite growth curve for Goliath Grouper could benefit from the inclusion of data from older fish or appropriate weighting (i.e., inversely weighted by sample size).

The larger size-at-age observed in Goliath Grouper from the Atlantic coast of Florida may suggest a density-dependent response to a lower population size due to its over-exploitation, or, alternatively, that fish in this region experience different environmental features (e.g., temperature) in concert with increased food availability, which can result in growth differences, such as seen in other fish species that occur on both coasts of Florida (e.g., Black Sea Bass) [41]. It will be important in the future to recapture and non-lethally age large Goliath Grouper from the east coast of Florida to provide a more robust sample size of older fish for the growth curve, although a non-lethal ageing method using either fin spines or rays may have some limitations when ageing fish older than 25 years ([47], this issue).

The differences in the age distribution and growth of Goliath Grouper from the Atlantic coast of Florida versus the GOM could not be attributed to different methods of ageing the fish, namely otoliths used by Bullock et al. [8] and rays used in this study. Notably, for the first time, the ray ageing method was directly validated for Goliath Grouper during our study using tagged and recaptured fish that were aged over multiple tag–recapture events. In addition, based on Carroll et al. ([47], this issue), the APE for ages determined from paired otolith and ray samples was 4.9% for the primary reader (DJM) for Goliath Grouper between 2 and 35 years of age, and the APE for between-reader precision for rays was 2.9%. In general, an APE < 5.5% indicates a highly precise or accurate method [76] and one that is therefore appropriate to use in an age-structured stock assessment. The use of rays or spines to age Goliath Grouper is not without difficulties, however, and the possibility that the Goliath Grouper sampled from the Atlantic coast of Florida in this study would now be 7 to 13 years older in 2023 (i.e., oldest 22-year-old fish surviving to be 35 years old) may require the use of spines with an applied methodological correction factor ([47], this issue).

5. Conclusions

Goliath Grouper from the Atlantic coast of Florida, which represent a significant proportion of the spawning population in the Southeastern USA, were shown conclusively to have the capacity to be protogynous hermaphrodites, and most likely diandric. However,

their sexual pattern appears to be functionally gonochoristic, based on their size, age, and sex ratio demographics, in combination with the low rate of known functional sex change. This makes them relatively more resilient to exploitation compared to strictly monandric protogynous hermaphrodites. It will be important to sample Goliath Grouper in the GOM on a greater sex-specific basis to determine if hermaphroditism occurs and at what level. The reproductive characteristics of fish can change based on the level of harvesting, such as their age and size at maturity, as well as potentially their reproductive strategies [34]. The former can be taken into account in stock assessments, especially by looking at changes over time. However, we have no knowledge of any study that has taken into account an actual change in the reproductive strategy of a species (i.e., functional gonochorism versus functional hermaphroditism), or the proportion of the population using one strategy versus another, over its exploitable life span.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes8080412/s1>, Table S1: Monthly numbers (*n*) and total lengths (mean and range) of Goliath Grouper sampled off the east coast of Florida during 2010–2016.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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References

1. Sadovy de Mitcheson, Y.; Craig, M.T.; Bertoni, A.A.; Carpenter, K.E.; Cheung, W.W.L.; Choat, J.H.; Cornish, A.S.; Fennessy, S.T.; Ferreira, B.P.; Heemstra, P.C.; et al. Fishing groupers towards extinction: A global assessment of threats and extinction risks in a billion dollar fishery. *Fish Fish.* **2013**, *14*, 119–136. [CrossRef]
2. Coleman, F.C.; Koenig, C.C.; Collins, L.A. Reproductive styles of shallow-water grouper (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. *Environ. Biol. Fish.* **1996**, *47*, 129–141. [CrossRef]
3. Sadovy, Y.; Eklund, A.M. *Synopsis of Biological Information on the Nassau Grouper, Epinephelus Striatus (Bloch 1792), and the Jewfish, E. itajara (Lichtenstein 1822)*; NMFS 146; U.S. Department of Commerce: Seattle, WA, USA, 1999; pp. 1–65.
4. Coleman, F.; Koenig, C.; Huntsman, G.; Musick, J.; Eklund, A.; McGovern, J.; Chapman, R.; Sedberry, G.; Grimes, C. Long-lived Reef Fishes: The Grouper-Snapper Complex. *Fisheries* **2000**, *25*, 14–21. [CrossRef]

5. Morris, A.; Roberts, C.; Hawkins, J. The threatened status of groupers (Epinephelinae). *Biodivers. Conserv.* **2000**, *9*, 919–942. [[CrossRef](#)]
6. Sadovy de Mitcheson, Y.; Colin, P.L.; Lindfield, S.J.; Bukurrou, A. A decade of monitoring an Indo-Pacific grouper spawning aggregation: Benefits of protection and importance of survey design. *Front. Mar. Sci.* **2020**, *7*, 571878. [[CrossRef](#)]
7. Heemstra, P.C.; Randall, J.E. *FAO Species Catalogue, Volume 16. Groupers of the World (Family Serranidae, Subfamily Epinephelinae). An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper and Lyretail Species Known to Date*; FAO: Rome, Italy, 1993.
8. Bullock, L.H.; Murphy, M.D.; Godcharles, M.F.; Mitchell, M.E. Age, growth, and reproduction of jewfish *Epinephelus itajara* in the eastern Gulf of Mexico. *Fish B-Noaa* **1992**, *90*, 243–249.
9. Bueno, L.S.; Bertoncini, A.A.; Koenig, C.C.; Coleman, F.C.; Freitas, M.O.; Leite, J.R.; De Souza, T.F.; Hostim-Silva, M. Evidence for spawning aggregations of the endangered Atlantic goliath grouper *Epinephelus itajara* in southern Brazil. *J. Fish Biol.* **2016**, *89*, 876–889. [[CrossRef](#)]
10. Koenig, C.C.; Bueno, L.S.; Coleman, F.C.; Cusick, J.A.; Ellis, R.D.; Kingon, K.; Locascio, J.V.; Malinowski, C.; Murie, D.J.; Stallings, C.D. Diel, lunar, and seasonal spawning patterns of the Atlantic goliath grouper, *Epinephelus itajara*, off Florida, United States. *Bull. Mar. Sci.* **2017**, *93*, 391–406. [[CrossRef](#)]
11. Sadovy, Y.; Dormeier, M. Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. *Coral Reefs* **2005**, *24*, 254–262. [[CrossRef](#)]
12. Frias-Torres, S. Habitat use of juvenile goliath grouper *Epinephelus itajara* in the Florida Keys, USA. *Endanger. Species Res.* **2006**, *2*, 1–6. [[CrossRef](#)]
13. Koenig, C.C.; Coleman, F.C.; Eklund, A.M.; Schull, J.; Ueland, J. Mangroves as essential nursery habitat for goliath grouper (*Epinephelus itajara*). *Bull. Mar. Sci.* **2007**, *80*, 567–585.
14. Gulf of Mexico Fishery Management Council (GMFMC). *Amendment 2 to the Reef Fish Fishery Management Plan*; Gulf of Mexico Fishery Management Council (GMFMC): Tampa, FL, USA, 1990.
15. South Atlantic Fishery Management Council (SAFMC). *Amendment Number 4, Regulatory Impact Review, Initial Regulatory Flexibility Analysis and Environmental Assessment for the Fishery Management Plan for the Snapper Grouper Fishery of the South Atlantic Region*; South Atlantic Fishery Management Council: Charleston, SC, USA, 1990; p. 200.
16. IUCN. *IUCN Red List of Threatened Animals*; IUCN: Gland, Switzerland, 1996.
17. Bertoncini, A.A.; Aguilar-Perera, A.; Barreiros, J.; Craig, M.T.; Ferreira, B.P.; Koenig, C. *The IUCN Red List of Threatened Species 2018: Epinephelus itajara*; IUCN: Gland, Switzerland, 2018; p. e.T195409A46957794.
18. Locatelli, A.C.P.; Bastos, R.F.; Oliveira, M.A.; Ferreira, B.P. Scientometric analysis and literature synthesis of 60 years of science on the Atlantic goliath grouper (*Epinephelus itajara*). *J. Fish. Biol.* **2023**, *102*, 740–756. [[CrossRef](#)]
19. Barreiros, J.P.; Coleman, F.C. West African Goliath Grouper: Where Are They between Senegal and Angola? *Fishes* **2023**, *8*, 318. [[CrossRef](#)]
20. Koenig, C.C.; Coleman, F.C.; Malinowski, C.R. Atlantic Goliath Grouper of Florida: To Fish or Not to Fish. *Fisheries* **2020**, *45*, 20–32. [[CrossRef](#)]
21. Cass-Calay, S.; Schmidt, T. *Standardized Catch Rates of Juvenile Goliath Grouper, Epinephelus itajara, from the Everglades National Park Creel Survey, 1973–1999*; SFD-2003-0016; National Marine Fisheries Service, Sustainable Fisheries Division: Miami, FL, USA, 2003.
22. Porch, C.E.; Eklund, A.M.; Scott, G.P. A catch-free stock assessment model with application to goliath grouper (*Epinephelus itajara*) off southern Florida. *Fish B-Noaa* **2006**, *104*, 89–101.
23. Koenig, C.C.; Coleman, F.C.; Kingon, K. Pattern of recovery of the Goliath Grouper *Epinephelus itajara* population in the southeastern US. *Bull. Mar. Sci.* **2011**, *87*, 891–911. [[CrossRef](#)]
24. SEDAR. *SEDAR 47 Stock Assessment Report: Southeastern U.S. Goliath Grouper*; SEDAR: North Charleston, SC, USA, 2016; p. 206.
25. Coleman, F.C.; Nunes, J.A.C.C.; Bertoncini, A.A.; Bueno, L.S.; Freitas, M.O.; Borgonha, M.; Leite, J.R.; Lima-Júnior, M.J.C.A.; Ferreira, B.; Bentes, B.; et al. Controversial opening of a limited fishery for Atlantic Goliath Grouper in the United States: Implications for population recovery. *Mar. Policy* **2023**, *155*, 105752. [[CrossRef](#)]
26. Kingsley, M.C.S. *The Goliath Grouper in Southern Florida: Assessment Review and Advisory Report. Report Prepared for the South Atlantic Fishery Management Council, the Gulf of Mexico Fishery Management Council, and the National Marine Fisheries Service*; REEF: Carlsbad, CA, USA, 2004; p. 17.
27. Murie, D.J.; Parkyn, D.C.; Koenig, C.C.; Coleman, F.C.; Schull, J.; Frias-Torres, S. Evaluation of finrays as a non-lethal ageing method for protected goliath grouper *Epinephelus itajara*. *Endanger. Species Res.* **2009**, *7*, 213–220. [[CrossRef](#)]
28. Collins, A.B.; Barbieri, L.R. *Behavior, Habitat, and Abundance of the Goliath Grouper, Epinephelus itajara, in the Central Eastern Gulf of Mexico*; NOAA Award Number NA07NMF4540085; Fish and Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission: St. Petersburg, FL, USA, 2010; p. 44.
29. Collins, A.B.; Barbieri, L.R.; McBride, R.S.; McCoy, E.D.; Motta, P.J. Reef relief and volume are predictors of Atlantic goliath grouper presence and abundance in the eastern Gulf of Mexico. *Bull. Mar. Sci.* **2015**, *91*, 399–418. [[CrossRef](#)]
30. Malinowski, C.R.; Perrault, J.R.; Coleman, F.C.; Koenig, C.C.; Stilwell, J.M.; Cray, C.; Stacy, N.I. The iconic Atlantic Goliath Grouper (*Epinephelus itajara*): A comprehensive assessment of health indices in the southeastern United States population. *Front. Vet. Sci.* **2020**, *7*, 635. [[CrossRef](#)] [[PubMed](#)]

31. Malinowski, C.R.; Stacy, N.I.; Coleman, F.C.; Cusick, J.A.; Dugan, C.M.; Koenig, C.C.; Ragbeer, N.K.; Perrault, J.R. Mercury offloading in gametes and potential adverse effects of high mercury concentrations in blood and tissues of Atlantic Goliath Grouper *Epinephelus itajara* in the southeastern United States. *Sci. Total Environ.* **2021**, *779*, 146437. [[CrossRef](#)] [[PubMed](#)]
32. Malinowski, C.; Coleman, F.; Koenig, C.; Locascio, J.; Murie, D. Are Atlantic goliath grouper, *Epinephelus itajara*, establishing more northerly spawning sites? Evidence from the northeast Gulf of Mexico. *Bull. Mar. Sci.* **2019**, *95*, 371–391. [[CrossRef](#)]
33. Armsworth, P.R. Effects of fishing on a protogynous hermaphrodite. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 568–578. [[CrossRef](#)]
34. Rhodes, K.; Taylor, B.; McIlwain, J. Detailed demographic analysis of an *Epinephelus polyphkadion* spawning aggregation and fishery. *Mar. Ecol. Prog. Ser.* **2011**, *421*, 183–198. [[CrossRef](#)]
35. Freitas, M.O.; Abilhoa, V.; Giglio, V.J.; Hostim-Silva, M.; de Moura, R.L.; Francini-Filho, R.B.; Minte-Vera, C.V. Diet and reproduction of the goliath grouper, *Epinephelus itajara* (Actinopterygii: Perciformes: Serranidae), in eastern Brazil. *Acta Ichthyol. Piscat.* **2015**, *45*, 1–11. [[CrossRef](#)]
36. Smith, C.L. A revision of the American groupers: *Epinephelus* and allied genera. *Am. Mus. Nat. Hist.* **1971**, *146*, 69–241.
37. Alonzo, S.H.; Ish, T.; Key, M.; Maccall, A.D.; Mangel, M. The importance of incorporating protogynous sex change into stock assessments. *Bull. Mar. Sci.* **2008**, *83*, 163–179.
38. Lowerre-Barbieri, S.; Menendez, H.; Bickford, J.; Switzer, T.S.; Barbieri, L.; Koenig, C. Testing assumptions about sex change and spatial management in the protogynous gag grouper, *Mycteroperca microlepis*. *Mar. Ecol. Prog. Ser.* **2020**, *639*, 199–214. [[CrossRef](#)]
39. Munday, P.L.; Kuwamura, T.; Kroon, F.J. Bidirectional sex change in marine fishes. In *Reproduction and Sexuality in Marine Fishes: Patterns and Processes*; Cole, K., Ed.; University of California Press: Berkeley, CA, USA, 2010.
40. Haddon, M. *Modelling and Quantitative Methods in Fisheries*; Chapman & Hall/CRC Press: New York, NY, USA, 2001.
41. Hood, P.B.; Godcharles, M.F.; Barco, R.S. Age, growth, reproduction, and the feeding ecology of Black Sea Bass, *Centropristis striata* (Pisces: Serranidae), in the eastern Gulf of Mexico. *Bull. Mar. Sci.* **1994**, *54*, 24–37.
42. Collins, A.B.; Barbieri, L. *An Evaluation of the Effects of Catch and Release Angling on Survival and Behavior of Goliath Grouper (Epinephelus itajara) with Additional Investigation into Residence and Long-Term Movement Patterns*; NOAA Award Number NA10NMF4330115; Fish and Wildlife Research Institute, Florida Fish & Wildlife Conservation Commission: St. Petersburg, FL, USA, 2014; p. 52.
43. Koenig, C.; Coleman, F.; Malinowski, C.; Ellis, R.D.; Murie, D.; Parkyn, D.C.; Friess, C.; Tzadik, O.E. *Regional age structure, reproductive biology and trophic patterns of adult Goliath Grouper in Florida*; NOAA Marine Fisheries Initiative (MARFIN) Project Final Report: Washington, DC, USA, 2016; p. 222.
44. Wallace, R.A.; Selman, K. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool.* **1981**, *21*, 325–343. [[CrossRef](#)]
45. Hunter, J.R.; Macewicz, B.J.; Lo, N.C.; Kimbrell, C.A. Fecundity, spawning, and maturity of female Dover Sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish B-Noaa* **1992**, *90*, 101–128.
46. Brown-Peterson, N.J.; Wyanski, D.M.; Saborido-Rey, F.; Macewicz, B.J.; Lowerre-Barbieri, S.K. A standardized terminology for describing reproductive development in fishes. *Mar. Coast. Fish.* **2011**, *3*, 52–70. [[CrossRef](#)]
47. Carroll, J.L.; Ellis, R.D.; Collins, A.B.; Murie, D.J. Dorsal fin spines and rays for nonlethal ageing of Goliath Grouper *Epinephelus itajara*. *Fishes* **2023**, *8*, 239. [[CrossRef](#)]
48. VanderKooy, S.; Carroll, J.; Elzey, S.; Gilmore, J.; Kipp, J. (Eds.) *A practical Handbook for Determining the Ages of Gulf of Mexico and Atlantic Coast Fishes*, 3rd ed.; Gulf States Marine Fisheries Commission and the Atlantic States Marine Fisheries Commission: Ocean Springs, MS, USA, 2020.
49. Beamish, R.J.; Fournier, D.A. A method for comparing the precision of a set of age determinations. *J. Fish. Res. Board Can.* **1981**, *34*, 982–983. [[CrossRef](#)]
50. Quist, M.C.; Pegg, M.A.; DeVries, D.R. Age and growth. In *Fisheries Techniques*, 3rd ed.; Zale, A.V., Parrish, D.L., Sutton, T.M., Eds.; American Fisheries Society: Bethesda, MD, USA, 2012; pp. 677–731.
51. Ogle, D.H.; Doll, J.C.; Wheeler, A.P.; Dinno, A. FSA: Simple Fisheries Stock Assessment Methods, R Package Version 0.9.4. 2023. Available online: <https://CRAN.R-project.org/package=FSA> (accessed on 3 February 2023).
52. Baty, F.; Ritz, C.; Charles, S.; Brutsche, M.; Flandrois, J.; Delignette-Muller, M. A toolbox for nonlinear regression in R: The package nlstools. *J. Stat. Softw.* **2015**, *66*, 1–21. [[CrossRef](#)]
53. Akaike, H. Information theory and an extension of the maximum likelihood principle. In *International Symposium on Information Theory*; Petrov, B.N., Csaki, F., Eds.; Akademiai Kiado: Budapest, Hungary, 1973; pp. 267–281.
54. Mazerolle, M.J. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c), R Package Version 2.3.2. 2023. Available online: <https://cran.r-project.org/package=AICcmodavg> (accessed on 3 February 2023).
55. Nelson, G.A. Fishery Science Methods and Models, R-Package Version 1.12-0. 2023. Available online: <https://cran.r-project.org/package=fishmethods> (accessed on 3 February 2023).
56. Brusher, J.H.; Schull, J. Non-lethal age determination for juvenile goliath grouper (*Epinephelus itajara*) from southwest Florida. *Endanger Species Res.* **2009**, *7*, 205–212. [[CrossRef](#)]
57. O'Hop, J.; Munyandorero, J. *SEDAR 47 Stock Assessment Report for Goliath Grouper of the South Atlantic and Gulf of Mexico, 2016*; FWC Report IHR2016-001, May 23, 2016; Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute: St. Petersburg, FL, USA, 2016; p. 109.
58. Sadovy, Y.; Shapiro, D.Y. Criteria for the diagnosis of hermaphroditism in fishes. *Copeia* **1987**, *1987*, 136–156. [[CrossRef](#)]

59. Fennessy, S.T.; Sadovy, Y. Reproductive biology of a diandric protogynous hermaphrodite, the serranid *Epinephelus andersoni*. *Mar. Freshw. Res.* **2002**, *53*, 147–158. [[CrossRef](#)]
60. Liu, M.; Sadovy, Y. The influence of social factors on juvenile sexual differentiation in a diandric, protogynous grouper *Epinephelus coioides*. *Ichthyol. Res.* **2011**, *58*, 84–89. [[CrossRef](#)]
61. Palma, P.; Takemura, A.; Libunao, G.X.; Superio, J.; de Jesus-Ayson, E.G.; Ayson, F.; Nocillado, J.; Dennis, L.; Chan, J.; Thai, T.Q.; et al. Reproductive development of the threatened giant grouper *Epinephelus lanceolatus*. *Aquaculture* **2019**, *509*, 1–7. [[CrossRef](#)]
62. Craig, M.T.; Hastings, P.A. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyol. Res.* **2007**, *54*, 1–17. [[CrossRef](#)]
63. Erisman, B.; Rosales-Casián, J.; Hastings, P. Evidence of gonochorism in a grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico. *Environ. Biol. Fish* **2008**, *82*, 23–33. [[CrossRef](#)]
64. Sadovy, Y.; Colin, P.L. Sexual development and sexuality in the Nassau Grouper. *J. Fish Biol.* **1995**, *46*, 961–976. [[CrossRef](#)]
65. Pears, R.J.; Choat, J.H.; Mapstone, B.D.; Begg, G.A. Demography of a large grouper, *Epinephelus fuscoguttatus*, from Australia's Great Barrier Reef: Implications for fishery management. *Mar. Ecol. Prog. Ser.* **2006**, *307*, 259–272. [[CrossRef](#)]
66. Collins, M.R.; Waltz, C.W.; Roumillat, W.A.; Stubbs, D.L. Contribution to the life history and reproductive biology of gag, *Mycteroperca microlepis* (Serranidae), in the South Atlantic Bight. *Fish B-Noaa* **1987**, *85*, 648–653.
67. Liu, M.; Sadovy, Y. Early gonadal development and primary males in the protogynous epinepheline, *Cephalopholis boenak*. *J. Fish Biol.* **2004**, *65*, 987–1002. [[CrossRef](#)]
68. Warner, R.R.; Robertson, D.R. *Sexual Patterns in the Labroid Fishes of the Western Caribbean, I: The Wrasses (Labridae)*; Smithsonian Institution Press: Washington, DC, USA, 1978; Volume 254.
69. Yoda, M.; Yoneda, M. Assessment of reproductive potential in multiple-spawning fish with indeterminate fecundity: A case study of yellow sea bream *Dentex hypselosomus* in the East China Sea. *J. Fish Biol.* **2009**, *74*, 2338–2354. [[CrossRef](#)]
70. Ferreri, R.; Barra, M.; Gargano, A.; Aronica, S.; Bonanno, A.; Genovese, S.; Rumolo, P.; Basilone, G. New evaluation of postovulatory follicle degeneration at high-temperature regimes refines criteria for the identification of spawning cohorts in the European Anchovy (*Engraulis encrasicolus*). *Animals* **2021**, *11*, 529. [[CrossRef](#)]
71. Mackie, M.C. Socially controlled sex-change in the half-moon grouper, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. *Coral Reefs* **2003**, *22*, 133–142. [[CrossRef](#)]
72. Kline, R.J.; Khan, I.A.; Holt, G.J. Behavior, color change and time for sexual inversion in the protogynous grouper (*Epinephelus adscensionis*). *PLoS ONE* **2011**, *6*, e19576. [[CrossRef](#)]
73. Kulaw, D.H.; Cowan, J.H., Jr.; Jackson, M.W. Temporal and spatial comparisons of the reproductive biology of northern Gulf of Mexico (USA) red snapper (*Lutjanus campechanus*) collected a decade apart. *PLoS ONE* **2017**, *12*, e0172360. [[CrossRef](#)]
74. Hixon, M.A.; Johnson, D.W.; Sogard, S.M. BOFFFFs: On the importance of conserving old-growth age structure in fishery populations. *ICES J. Mar. Sci.* **2013**, *71*, 2171–2185. [[CrossRef](#)]
75. Barneche, D.R.; Robertson, D.R.; White, C.R.; Marshall, D.J. Fish reproductive-energy output increases disproportionately with body size. *Science* **2018**, *360*, 642–645. [[CrossRef](#)]
76. Campana, S.E. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* **2001**, *59*, 197–242. [[CrossRef](#)]

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