Marine Resources Monitoring, Assessment and Prediction Program: Report on South Atlantic Gray Triggerfish, *Balistes capriscus*, for the SEDAR 32 Data Workshop

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SEDAR32-DW-05

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***Addendum added to reflect changes made during the data workshop.**
Final data is found in the addendum.

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Introduction

Gray triggerfish (*Balistes capriscus*) is a marine species in the family Balistidae that occurs in the tropical and temperate zones across the entire Atlantic Ocean, including the Mediterranean Sea (Bernardes 2002, Robins and Ray 1986). Gray triggerfish occur in coastal waters of the western Atlantic from Nova Scotia (Canada) to Argentina, including the Gulf of Mexico and off Bermuda (Bernardes 2002, Robins and Ray 1986). Throughout this distribution gray triggerfish generally are found at depths of 0-100 m (Harmelin-Vivien and Quéro 1990). In the Gulf of Mexico, they are found commonly at depths between 12 and 42 m among reefs and hard bottom habitat (Harper and McClellan 1997).

Gray triggerfish are iteroparous gonochorists, building nests and exhibiting bi-parental care (Mackican and Szedlemayer 2007). Early life stages include demersal eggs and pelagic larvae (Richards and Lindeman 1987). Eggs may not fully hydrate or exhibit the degree of yolk fusion observed in pelagic eggs (Moore 2001). Postovulatory follicles (POFs) are rare in collections possibly due to reduced feeding by spawning females, thereby reducing the chances of females foraging, accepting bait and interacting with collection gear at this phase of the reproductive cycle (Moore 2001). It is unknown if fecundity is determinate or indeterminate. Thus, we know little about female reproductive potential, spawning frequency, and overall ovarian organization.

Male gray triggerfish have separate, small, oval-shaped testes that lie close together on the ventral side of the swim bladder. The common spermatic duct is lined with columnar secretory epithelial cells and surrounded by an accessory gland that may function to secrete substances that maintain spermatozoa while they are stored. Spermatic ducts act as a storage system for spermatozoa before release; therefore, both the testes and the spermatic duct/accessory gland complex are needed to accurately assess reproductive condition. A sample from the testes or duct/gland alone is usually only useful to assess sexual maturity (i.e., juveniles vs. adult).

Previous research on the age and growth of gray triggerfish has been derived predominately from fish outside the jurisdiction of the South Atlantic Fisheries Management Council (SAFMC). Peer-reviewed and unpublished studies in other regions, using the first dorsal spine as the aging structure, include the southern coast of Africa (Caveriviere et al. 1981, Ofori-Danson 1989, Aggrey-Fynn 2009), Brazil (Bernardes 2002), and the Gulf of Mexico (Johnson and Saloman 1984, Wilson et al. 1995, Hood and Johnson 1997, Ingram 2001, Fioramonti 2012). Along the US South Atlantic, only two of these have focused on the age and growth of gray triggerfish in coastal waters (Escorriola 1991, Moore 2001). Moore (2001) found that gray triggerfish collected among reefs and hard bottom habitat from Cape Fear, North Carolina to Cape Canaveral, Florida ranged in age from 0 to 10 years old, with a maximum observed fork length (FL) of 560 mm. Moore (2001) also found that males were significantly larger than females. To our knowledge, all previous studies conducted on the age and growth of gray triggerfish utilized the first dorsal spine as the primary aging structure. The spine is used rather than the otoliths due to the extremely small size and irregular shape of gray triggerfish otoliths. This makes routine extraction and examination of otoliths in this species difficult and time consuming compared to other species. Currently, no published documentation exists of comparisons among potential aging structures (spines, otoliths, vertebrae, etc.) in gray triggerfish.

Gray triggerfish from the US South Atlantic are undergoing an inaugural benchmark stock assessment through the SouthEast Data, Assessment, and Review (SEDAR) process in 2013 (SEDAR 32). This assessment will include data through 2011.

This report describes the fishery independent data collected by the Marine Resources Monitoring, Assessment, and Prediction (MARMAP), Southeast Atlantic Monitoring, Assessment, and Prediction (SEAMAP-SA) and Southeast Fishery Independent Survey (SEFIS) programs (for details of these programs see below and Ballenger et al. 2011).

Methods

Spines and gonadal tissues were taken from gray triggerfish specimens collected from coastal and offshore waters between Cape Lookout, North Carolina, and Key West, Florida, between 1973-2011 (N=8,607). The vast majority of specimens were collected during standard sampling by the MARMAP program (fishery-independent, Project ID: P05, P55, & Q26) and using chevron traps (gear code 324), but over the years other gears collected gray triggerfish such as Florida traps (gear code 074), blackfish traps (gear code 053), mini-Antillean "S" traps (gear code 041), 3/4 scale Yankee trawl (gear code 022), snapper/bandit reel (gear code 043) and hook and line (gear code 014), spear gun (gear code 065), Experimental trap (gear code 073), and Lionfish trap (gear code 540) (Collins 1990, Harris and McGovern 1997, Harris et al. 2004, MARMAP 2009). SEFIS also provided samples using chevron traps and hook and line since 2010. Thirty eight gray triggerfish were collected during standard sampling by the SEAMAP-SA program (fishery-independent, Project P94), using Mongoose-type Falcon trawl (gear code 233). Gray triggerfish specimens were also obtained from commercial catches (fishery-dependent, Project ID: P50) using hook and line (gear code 014), dip net (gear code 019), snapper/bandit reel (gear code 043), and chevron trap (gear code 324).

Workshops were held in Charleston SC (September 2011) and NOAA SEFSC-Beaufort Laboratory (October 2012) in preparation for SEDAR 32. The goals of the workshops were to (1) compare sample preparation, reading methods and data analysis of the first dorsal spine of gray triggerfish, with an emphasis on addressing difficulties and issues previously encountered by Gulf of Mexico and Atlantic labs and (2) compare reproductive histological assessments and finalize methodology and analyses (see SEDAR DW-03 for results).

After collection, catches were sorted by species and processed following standard protocols (see details in MARMAP 2009). Whole gray triggerfish were weighed to the nearest gram (g) and total length (TL), fork length (FL), and standard length (SL) were measured to the nearest mm. Note that fork length was used in all length-based analyses in this report based on the SEDAR 32 Scoping Conference Call. Spines were removed from all fish and stored dry prior to processing. Samples of gonad tissues were removed and stored in 11 seawater formalin until later processing.

Age

Spine sections were processed using standard methods as discussed and agreed upon by various collaborating fish aging labs that are providing age data to SEDAR 32 (SEDAR32-DW03).
MARMAP utilized transverse sections of the dorsal spine immediately distal to the condyle groove for age determination. Spines were cleaned to a degree that surplus skin and muscle tissue was removed prior to sectioning. An Isomet low-speed saw was used to cut 0.4-0.7 mm thick sections from gray triggerfish spines. The workshop concluded that the increments as identified by the workshop participants can be considered annuli, and can be used to determine the age of gray triggerfish.
At SC-DNR, spine sections were examined independently by two readers and re-examined jointly when differences in age estimation occurred. Aging was done without knowledge of specimen length or date of capture. If disagreement persisted, the specimen was eliminated from age analyses. In addition, we recorded quality and edge type (Table 1).

Based on evidence for a June-July spawning peak in females (Figure 1), the workshop recommended the use of the following criteria to convert increment counts to annual ages: any fish captured prior to July 1st with an edge type of 3 or 4 were assigned a calendar age of increment count plus one, otherwise calendar age equals increment count. Calendar ages were used in analyses of sex ratio, male and female age at maturity, age compositions, and spawning periodicity. Fractional ages were assigned based on calendar age and adjusted for date of capture assuming a July 1 birthday. Fractional age was used in growth models, age and depth, and fecundity analyses.

Reproduction

Following capture and dissection, the posterior portion of the gonads were fixed for 7–14 d in 11% seawater–formalin solution buffered with marble chips and transferred to 50% isopropanol for an additional 7–14 d. Male gray triggerfish are unique in that both testes and the spermatic duct/accessory gland must be collected for complete analysis. For this reason, two different sections of the spermatic duct/accessory gland were taken along with a sample of the testes to ensure accurate staging. Reproductive tissue was processed in an automated and self-enclosed tissue processor and blocked in paraffin. Three transverse sections (6–8 um thick) were cut from each sample with a rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counterstained with eosin-y. Sections were viewed under a compound microscope at 20-400X magnification, and sex and reproductive class were determined without knowledge of capture date, specimen length, or specimen age. Descriptive criteria for reproductive classes with the inclusion of subclasses for male staging was outlined and recommended during the gray triggerfish workshops (Table 2 and Table 3). Three readers independently determined sex and reproductive state using histological criteria (Moore 2001, Wyanski 2006, and Brown-Peterson et al. 2011). When assignments differed, the readers re-examined the section simultaneously to determine reproductive state. Females were considered to be in spawning condition if they possessed oocytes undergoing maturation (i.e., fusing of yolk globules, germinal vesicle migration and breakdown) or postovulatory follicles (POFs).

Analyses

All analyses were done using "R". In some instances the data set was subdivided based on depth, latitudinal and temporal state. The following criteria was used during for these analyses:

Depth:

Inshore: Samp_depth < 30m;
Offshore: Samp_depth >= 30m

Latitude:

South: Latitude < 32 degrees;
North: Latitude >= 32 degrees

Period:

Early: Year<1990;
Mid : 1989 < Year < 2000;
Late: Year>1999

Length/length and length/weight analyses were done using linear regression analyses with all length in mm and weight in grams. As we have no gutted weight data available, no weight/weight analyses were done. Sex ratio data were analyzed using a Chi-square goodness of fit test to determine if these ratios differed among size classes from an expected 1:1 (Zar 1984). R Statistical Software was used to estimate length at 50% maturity (L50) and age at 50% maturity (A50). Workshop participants also recommended using gonad weight versus FL and whole fish weight as a proxy for a fecundity estimate. The R2 values are adjusted for degrees of freedom.

Results

Gray triggerfish analyzed for this report were captured between latitude 27.23^0 and 35.10^0 and at a depth range of 0 to 93 meters. Specimens ranged in fork length from 75 to 578 mm and ranged in weight from 11 to 5,000 g. Ages ranged from 0 to 13.

Length/length and length/weight conversions.

Linear regression analyses indicated that there were no significant differences in the slopes of various length/length regressions between males and females (Table 4) or in the slopes of length (mm) versus weight (g) between males and females (Table 5, Figure 2). In both cases, assuming equal slopes, there was a significant different in intercepts between males and females. However, these differences were a result of a large data set and have no biological relevance. Our recommendation is to use conversion equations for males and females combined (Table 4).

Length-age data from males, females and all data was fitted to the von Bertalanffy growth model (FL = Linf*exp(-k*(age-t_0) to generate estimates of growth parameters for gray triggerfish (Table 6, Figure 3 and 4). The results show differences in growth rate between the sexes and our recommendation is to use sex-specific growth Von Bertalanffy parameters.

Reproduction

There was a high degree of overlap in the length distributions of definitely mature and regenerating gray triggerfish and modest overlap in the lengths of immature and all mature individuals, indicating that individuals were correctly assigned to the immature and regenerating classes (Figures 5 & 6).

The results of all modeling indicate that gray triggerfish mature before they reach one year of age. Age based maturity analyses were done using calendar year. Size (1 cm size bins) and age (by year) at maturity was based on a Logit model, as it provided the best fit. Female size and age at maturity yielded an A50 = 0.26 yr and L50 = 173mm (Table 7, 8, and 9). Male length and age at maturity yielded A50 = 0.66 yr and L50 = 181mm (Table 10, 11, and 12).

The overall sex ratio of 1.208 was significantly different form a 1:1 ratio, with the proportion of females being greater than that for males (Table 13). However, analyses also indicated that female gray triggerfish were more abundant than males at smaller sizes, while and male gray triggerfish were significantly more abundant at sizes $\geq$ 400mm FL (Table 14, Table 15, Figure 7, Figure 8).

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Table 1. Spine edge type and quality.

EDGE TYPE

Code	Description	
1	Opaque zone on the edge.	
2	Narrow translucent zone on edge	Width less than about 30% of previous increment
3	Medium translucent zone on edge	Width about 30-60% of previous increment
4	Wide translucent zone on edge	Width more than about 60% of previous increment

READABILITY

Code		Description and analysis consequence
A	Unreadable	Omit otolith from analysis
B	Very difficult to read	Age estimate between readers are expected to be >2 year for young, and > 4 yrs for old fish (>10 yrs) Agreement on age may be difficult to reach, in which case otoliths should be classified as A and omitted from the analysis.
C	Fair readability	Age estimates between readers should be within 2 year in young, and within 4 years in old fish (>10 yrs). Agreement after second reading is expected after some discussion.
D	Good readability	Age estimates between readers should be within 1 year for young, to 2 years in old fish (> 10 years). Agreement after second reading is expected without much discussion.
E	Excellent readability	Age estimates between readers should be the same.

Table 2: Histological interpretation of female gray triggerfish. Most descriptors based on Moore (2001), Wyanski (2006) and Brown-Peterson et al. (2011).

Maturity Class	**Description**
Uncertain Maturity (Class 0)	Inactive ovaries, primary growth oocytes only; unable to assess maturity
Immature (Class 1)	Primary growth oocytes 20-60 micron diameter (Moore 2001); no evidence of atresia. In comparison to regenerating female, transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, oogonia abundant along margin of lamellae, ovarian wall is thinner
Cortical alveolar oocytes (Class E)	Early Developing; Previtellogenic; cortical alveolar oocytes 140-200 micron diameter
Yolked oocytes (Class F)	Vitellogenic; Most advanced oocytes in yolk-granule or yolk-globule stage; oocyte 170-400 micron diameter
Migratory nucleus oocytes (Class G)	Oocyte maturation; partial coalescence of yolk globules possible; Oocytes 385-500 micron diameter
Postovulatory follicles (POFs): early (Class B), intermediate (Class C), late (Class D)	Vitellogenic oocytes and POFs; Evidence of recent spawn; note that beta-stage atresia cannot always be distinguished from medium to old postovulatory follicles (Hunter and Macewicz 1985)
Regressing (Class 4)	>50% of yolked oocytes undergoing alpha or beta stage of atresia
Regenerating (Class 5)	Primary growth oocytes > 60 micron 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
Mature specimen (Class 8)	Mature, but postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive class
Unknown (Class 9)	Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state

Table 3: Histological interpretation of male gray triggerfish. Most descriptors based on Moore (2001), Wyanski (2006) and Brown-Peterson et al. (2011) with the inclusion of sub-classes.

Maturity Class	Sub-Class	Description
Uncertain Maturity (Class 0)		Inactive testes; unable to assess maturity
Immature (Class 1)		Small transverse section compared to regenerating male; little or no spermatocyte development
Developing (Class 2)		Limited spermatogenesis in testes; elongation of lobules and some accumulation of spermatozoa (SZ) in testes BUT no accumulation in lobules, efferent ducts (within testes), and spermatic ducts
Spawning Capable (3 sub-classes)	Early Spawning Capable (Subclass ESC)	Spermatozoa evident in ducts; amount of spermatogenesis in testes ranges from limited to extensive; in ducts, greater area of structural tissue compared to sinuses
	Storage (Subclass H)	Spermatozoa storage within expanding ducts; >50% of area of sinuses densely packed with spermatozoa; amount of spermatogenesis in testes ranges from limited to extensive
	Recent Spawn (Subclass 7)	Large, expanded ducts not as densely packed with spermatozoa; area of sinuses greater than that of structural tissue; usually has empty lobules toward center of testes
Regressing (Class 4)		Limited spermatogenesis in testes; shrinking ducts/lobules with residual spermatozoa present; overall number of ducts containing spermatozoa also small; increase of connective tissue in testes, proliferating from center; may have enlarged cells lining sinuses
Regenerating (Class 5)		Larger transverse section compared to immature male; very limited or no spermatogenesis in testes; little or no residual spermatozoa in ducts
Mature Specimen (Class 8)		Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive class
Unknown (Class 9)		Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state

Table 4. Gray Triggerfish length versus length relationships. TL=total length, FL=fork length, SL=stndard length. All lengths are in mm. n=number of combinations available and used for analyses. R2 is adjusted for degrees of freedom.

	equation	n	a	b	R2
All	TL=a+b*FL	8543	-17.347	1.208	0.9677
	TL=a+b*SL	8541	2.059	1.381	0.9530
	FL=a+b*SL	8591	16.173	1.144	0.9833
Males	TL=a+b*FL	3621	-19.616	1.215	0.9657
	TL=a+b*SL	3618	1.504	1.388	0.9514
	FL=a+b*SL	3652	17.722	1.141	0.9835
Females	TL=a+b*FL	4376	-15.005	1.200	0.9633
	TL=a+b*SL	4375	4.832	1.369	0.9451
	FL=a+b*SL	4391	16.927	1.139	0.9791

Table 5. Length (mm) versus weight (g) relationships. FISH_WT=fish wet weight in grams, TL=total length, FL=fork length, SL=standard length. All lengths are in mm. n=number of combinations available and used for analyses. R2 is adjusted for degrees of freedom.

	equation	n	a	b	R2
All	Log (WT) = log a + bTL	8522	-9.570	2.742	0.9614
	Log (WT) = log a + bFL	8571	-10.359	2.946	0.9852
	Log (WT) = log a + bSL	8571	-9.046	2.810	0.9753
Males	Log (WT) = log a + bTL	3611	-9.592	2.744	0.9587
	Log (WT) = log a + bFL	3646	-10.463	2.962	0.9861
	Log (WT) = log a + bSL	3644	-9.100	2.818	0.9758
Females	Log (WT) = log a + bTL	4366	-9.613	2.751	0.9575
	Log (WT) = log a + bFL	4381	-10.387	2.953	0.9826
	Log (WT) = log a + bSL	4381	-0.9033	2.808	0.9706

Table 6. Estimates of Von Bertalanffy growth parameters base on non-linear regression analysis using available total length (mm) and age (fractional age) data. N=number of aged fish used in analysis. Linf= asymptotic FL, SE= standard error, K= Von Bertalanffy growth parameter. T0= VonBertanffy parameter for age at theoretical length=0.

	n	Linf	SE	k	SE	t0	SE
All Fish	7,392	407	3.33	0.3104	0.01152	-0.78	0.0499
Males	3,208	435	5.84	0.2916	0.01680	-0.79	0.1286
Females	3,981	382	3.51	0.3353	0.01530	-0.80	0.1045

Table 7. Results of various regression model analyses for age and length at maturity for female gray triggerfish. Data for all years and all gears were combined. Age is expressed in fractional age and length is fork length in mm. N=number of fish used in analyses, a/lamda= coefficient, b/k= coefficient, A50= age at 50% maturity, L50=length at 50% maturity. AIC=Akaike's Information Criterion. Parameters in bold represent the best fit models.

	Model	N	a/lamda	SE	b/k	SE	A50/L50	AIC
Age	**Logistic Logit**	**3861**	**0.344**	**0.136**	**0.435**	**0.040**	**0.257**	**678.30**
	Logistic Probit	3861	0.344	0.136	0.435	0.040	-0.791	700.65
	Logistic - clog-log	3861	0.394	0.096	0.233	0.025	-1.688	723.22
	Logistic - Cauchy	3861	-5.347	0.760	5.659	0.701	0.945	695.83
	Gompertz	3861	2.071	0.293	1.260	0.059	0.95	-4086.46
Length	**Logistic Logit**	**4251**	**-9.834**	**0.829**	**0.057**	**0.004**	**172.89**	**404.70**
	Logistic Probit	4251	-4.246	0.375	0.025	0.002	167.27	430.91
	Logistic - clog-log	4251	-2.330	0.261	0.014	0.001	164.66	488.51
	Logistic - Cauchy	4251	-41.110	5.450	0.238	0.031	172.85	472.11

Table 8. Female fork length (mm) at maturity using Logistic-Logit. % Mature= Percent Mature, 0.5= length (fork length in mm) at maturity, Prop. Mat= projected proportion mature using Logit model.

					Logistic	Logit
Length	**Immature**	**Mature**	**Total**	**% Mature**	**0.5**	**Prop. Mat**
8	2	0	2	0.000	173	0.005
9	0	0	0	NA	173	0.009
10	3	0	3	0.000	173	0.016
11	2	0	2	0.000	173	0.027
12	1	0	1	0.000	173	0.047
13	3	0	3	0.000	173	0.080
14	8	1	9	0.111	173	0.133
15	9	1	10	0.100	173	0.214
16	17	3	20	0.150	173	0.324
17	17	15	32	0.469	173	0.459
18	9	23	32	0.719	173	0.600
19	9	30	39	0.769	173	0.726
20	8	46	54	0.852	173	0.824
21	5	45	50	0.900	173	0.892
22	9	73	82	0.890	173	0.936
23	1	66	67	0.985	173	0.963
24	4	110	114	0.965	173	0.978
25	0	104	104	1.000	173	0.988
26	0	155	155	1.000	173	0.993
27	0	123	123	1.000	173	0.996
28	0	225	225	1.000	173	0.998
29	0	203	203	1.000	173	0.999
30	1	327	328	0.997	173	0.999
31	1	272	273	0.996	173	1.000
32	0	340	340	1.000	173	1.000
33	0	264	264	1.000	173	1.000
34	0	322	322	1.000	173	1.000
35	0	265	265	1.000	173	1.000
36	0	317	317	1.000	173	1.000
37	0	203	203	1.000	173	1.000
38	0	206	206	1.000	173	1.000
39	1	134	135	0.993	173	1.000
40	0	116	116	1.000	173	1.000
41	0	63	63	1.000	173	1.000
42	0	38	38	1.000	173	1.000
43	0	22	22	1.000	173	1.000
44	0	12	12	1.000	173	1.000

45	0	6	6	1.000	173	1.000
46	0	5	5	1.000	173	1.000
47	0	3	3	1.000	173	1.000
48	0	1	1	1.000	173	1.000
49	0	1	1	1.000	173	1.000
50	0	0	0	NA	173	1.000
51	0	0	0	NA	173	1.000
52	0	0	0	NA	173	1.000
53	0	0	0	NA	173	1.000
54	0	0	0	NA	173	1.000
55	0	0	0	NA	173	1.000
56	0	1	1	1.000	173	1.000

Table 9. Female age at maturity using Logistic-Logit. Female age at maturity using Logistic-Logit. % Mature= Percent Mature, 0.5= age at maturity, Prop. Mat= projected proportion mature using Logit model. Calendar age was used in these analyses.

Age	Immature	Mature	Total	% Mature	Logistic 0.50	Logit Prop. Mat
0	4	1	5	0.200	0.26	0.428
1	23	39	62	0.629	0.26	0.697
2	32	212	244	0.869	0.26	0.876
3	20	564	584	0.966	0.26	0.956
4	8	846	854	0.991	0.26	0.985
5	1	821	822	0.999	0.26	0.995
6	3	612	615	0.995	0.26	0.998
7	2	362	364	0.995	0.26	0.999
8	1	177	178	0.994	0.26	1.000
9	1	85	86	0.988	0.26	1.000
10	0	31	31	1.000	0.26	1.000
11	0	10	10	1.000	0.26	1.000
12	0	3	3	1.000	0.26	1.000
13	0	3	3	1.000	0.26	1.000

Table 10. Results of various regression model analyses for age and length at maturity for male gray triggerfish. Data for all years and all gears were combined. Age is expressed in fractional age and length is fork length in mm. n=number of fish used in analyses, a/lamda= coefficient, b/k= coefficient , A50= age at 50% maturity, L50=length at 50% maturity. AIC=Akaike's Information Criterion. Parameters in bold represent the best fit models.

	Model	N	a/lamda	SE	b/k	SE	A50/L50	AIC
Age	**Logistic Logit**	**3163**	**-0.827**	**0.281**	**1..261**	**0.104**	**0.66**	**596.46**
	Logistic Probit	3163	-0.010	0.146	0.525	0.046	0.02	612.99
	Logistic - clog-log	3163	0.074	0.110	0.313	0.031	-0.24	630.16
	Logistic - Cauchy	3163	-5.380	0.707	5.183	0.640	1.04	615.57
	Gompertz	3163	5.292	0.864	1.543	0.075	0.96	-3006.48
Length	**Logistic Logit**	**3602**	**-9.019**	**0.825**	**0.050**	**0.004**	**180.66**	**434.99**
	Logistic Probit	3602	-4.402	0.426	0.025	0.002	177.84	440.52
	Logistic - clog-log	3602	-3.438	0.375	0.018	0.002	188.81	454.06
	Logistic - Cauchy	3602	-30.938	3.898	0.168	0.021	183.86	509.53

Table 11. Male fork length (mm) at maturity using Logistic Logit. % Mature= Percent Mature, 0.5= length (fork length in mm) at maturity, Prop. Mat= projected proportion mature using Logit model.

					Logistic Logit	
Length	Immature	Mature	Total	% Mature	0.5	Prop. Mat
13	1	0	1	0.000	181	0.074
14	4	0	4	0.000	181	0.116
15	6	0	6	0.000	181	0.178
16	8	2	10	0.200	181	0.263
17	16	6	22	0.273	181	0.370
18	14	18	32	0.563	181	0.492
19	17	24	41	0.585	181	0.615
20	12	39	51	0.765	181	0.724
21	5	36	41	0.878	181	0.812
22	6	65	71	0.915	181	0.877
23	2	42	44	0.955	181	0.922
24	5	80	85	0.941	181	0.951
25	5	66	71	0.930	181	0.970
26	1	65	66	0.985	181	0.981
27	2	79	81	0.975	181	0.989
28	2	99	101	0.980	181	0.993
29	1	101	102	0.990	181	0.996
30	0	144	144	1.000	181	0.997
31	0	140	140	1.000	181	0.998
32	0	187	187	1.000	181	0.999
33	0	177	177	1.000	181	0.999
34	0	259	259	1.000	181	1.000
35	0	156	156	1.000	181	1.000
36	0	266	266	1.000	181	1.000
37	0	177	177	1.000	181	1.000
38	0	207	207	1.000	181	1.000
39	0	148	148	1.000	181	1.000
40	0	205	205	1.000	181	1.000
41	0	152	152	1.000	181	1.000
42	0	178	178	1.000	181	1.000
43	0	99	99	1.000	181	1.000
44	0	90	90	1.000	181	1.000
45	0	66	66	1.000	181	1.000
46	0	42	42	1.000	181	1.000
47	0	24	24	1.000	181	1.000
48	0	26	26	1.000	181	1.000
49	0	8	8	1.000	181	1.000

50	0	9	9	1.000	181	1.000
51	0	7	7	1.000	181	1.000
52	0	0	0	NA	181	1.000
53	0	3	3	1.000	181	1.000
54	0	2	2	1.000	181	1.000
55	0	0	0	NA	181	1.000
56	0	0	0	NA	181	1.000
57	0	0	0	NA	181	1.000
58	0	1	1	1.000	181	1.000

Table 12. Male age at maturity using Logistic Logit. % Mature= Percent Mature, 0.5= age at maturity, Prop. Mat= projected proportion mature using Logit model. Calendar age was used in these analyses.

					Logistic	Logit
Age	**Immature**	**Mature**	**Total**	**% Mature**	**0.50**	**Prop. Mat**
0	1	0	1	0.000	0.66	0.304
1	29	27	56	0.482	0.66	0.607
2	35	200	235	0.851	0.66	0.845
3	10	410	420	0.976	0.66	0.951
4	10	651	661	0.985	0.66	0.985
5	4	665	669	0.994	0.66	0.996
6	3	527	530	0.994	0.66	0.999
7	1	305	306	0.997	0.66	1.000
8	0	180	180	1.000	0.66	1.000
9	0	71	71	1.000	0.66	1.000
10	0	21	21	1.000	0.66	1.000
11	0	9	9	1.000	0.66	1.000
12	0	3	3	1.000	0.66	1.000
13	0	1	1	1.000	0.66	1.000

Table 13. Gray triggerfish overall sex ratio.

	Ratio: Female:Male	# Male	# Female	Proportion Female	Chi-squared	P-value
Overall	1.208	3550	4287	0.547	69.308	<0.0001

Table 14. Length based sex ratio by one centimeter bins.

Fork Length	Female:Male	# Male	# Female	Proportion	Chi-square	P-value
14	n/a	0	2	1.000	n/a	n/a
15	2.000	1	2	0.667	n/a	n/a
16	2.800	5	14	0.737	4.263	0.39
17	4.167	6	25	0.806	11.645	0.001
18	1.909	22	42	0.656	6.250	0.012
19	1.467	30	44	0.595	2.649	0.104
20	1.444	45	65	0.591	3.636	0.057
21	1.357	42	57	0.576	2.273	0.132
22	1.179	67	79	0.541	0.986	0.321
23	1.553	47	73	0.608	5.633	0.018
24	1.550	80	124	0.608	9.490	0.002
25	1.580	69	109	0.612	8.989	0.003
26	2.333	69	161	0.700	36.800	<0.0001
27	1.638	80	131	0.621	12.327	<0.0001
28	2.216	102	226	0.689	46.878	<0.0001
29	2.000	102	204	0.667	34.000	<0.0001
30	2.278	144	328	0.695	71.729	<0.0001
31	1.943	140	272	0.660	42.291	<0.0001
32	1.824	187	341	0.646	44.917	<0.0001
33	1.470	181	266	0.595	16.163	<0.0001
34	1.242	260	323	0.554	6.808	0.009
35	1.699	156	265	0.629	28.221	<0.0001
36	1.192	266	317	0.544	4.461	0.035
37	1.140	178	203	0.533	1.640	0.2
38	0.995	207	206	0.499	0.002	0.961
39	0.905	148	134	0.475	0.695	0.405
40	0.568	206	117	0.362	24.523	<0.0001
41	0.412	153	63	0.292	37.500	<0.0001
42	0.213	178	38	0.176	90.741	<0.0001
43	0.222	99	22	0.182	49.000	<0.0001
44	0.133	90	12	0.118	59.647	<0.0001
45	0.091	66	6	0.083	50.000	<0.0001
46	0.119	42	5	0.106	29.128	<0.0001

47	0.167	24	4	0.143	14.286	<0.0001
48	0.037	27	1	0.036	24.143	<0.0001
49	0.125	8	1	0.111	n/a	n/a
50	0.000	9	0	0.000	n/a	n/a
51	0.000	7	0	0.000	n/a	n/a
53	0.000	3	0	0.000	n/a	n/a
54	0.000	2	0	0.000	n/a	n/a
56	n/a	0	1	1.000	n/a	n/a
58	0.000	1	0	0.000	n/a	n/a

Table 15. Age based sex ratio by one centimeter fork length bins.

	Female:Male	# Male	# Female	Proportion	Chi-square	P-value
0	n/a	0	1	1.0000	n/a	n/a
1	1.778	36	64	0.6400	7.84	0.0051
2	1.223	211	258	0.5501	4.71	0.03
3	1.415	417	590	0.5859	29.721	<0.0001
4	1.305	660	861	0.5661	26.562	<0.0001
5	1.238	669	828	0.5531	16.888	<0.0001
6	1.164	531	618	0.5379	6.588	0.01
7	1.175	308	362	0.5403	4.352	0.037
8	0.983	180	177	0.4958	0.025	0.874
9	1.211	71	86	0.5478	1.433	0.231
10	1.409	22	31	0.5849	1.528	0.216
11	1.111	9	10	0.5263	0.053	0.819
12	1.000	3	3	0.5000	n/a	n/a
13	3.000	1	3	0.7500	n/a	n/a

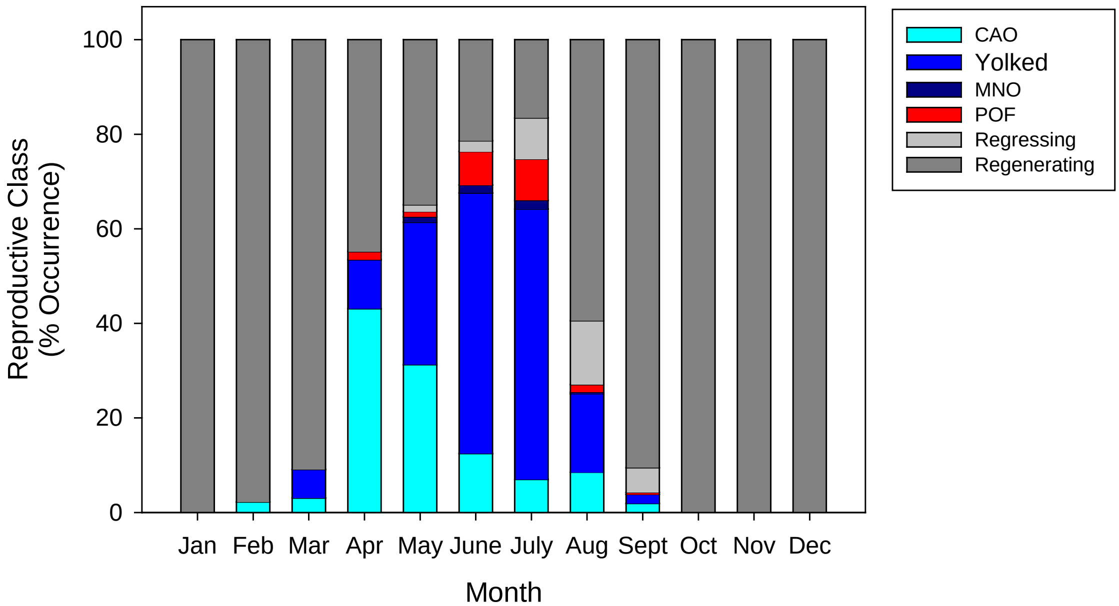


Figure 1. Female gray triggerfish spawning seasonality. CAO= cortical alveolar oocytes, Yolked= Yolked oocytes, MNO= migratory nuclear oocytes, POF= post ovulatory ooctyes.

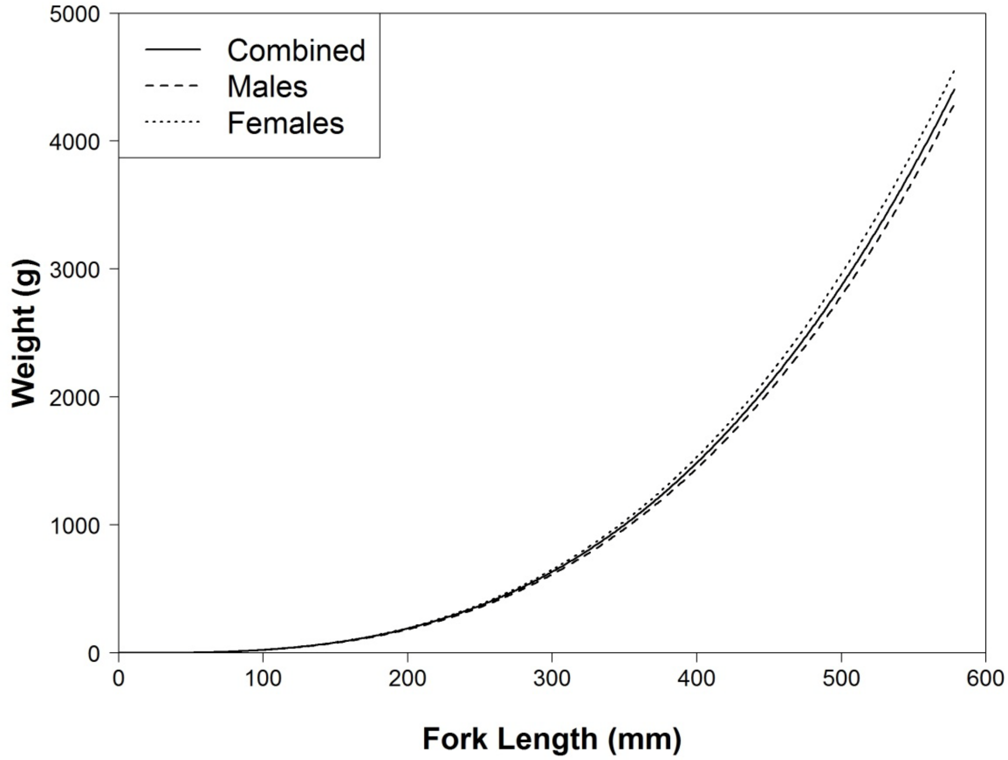


Figure 2. Regression lines of analyses of gray triggerfish fork length (mm) versus whole wet weight (g).

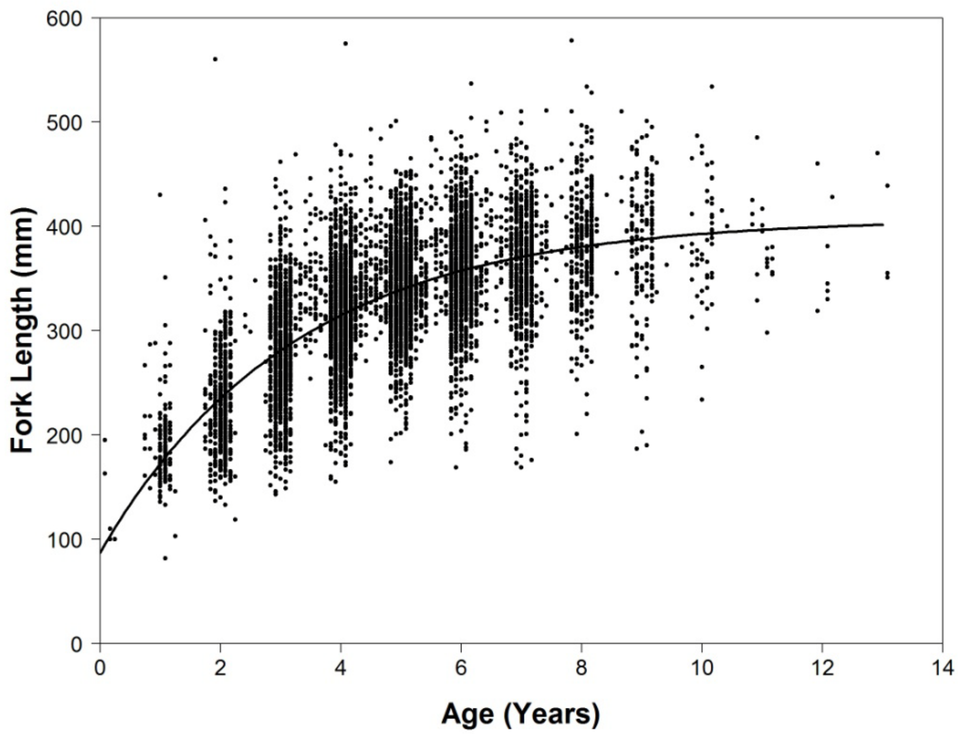


Figure 3. Gray triggerfish Von Bertalanffy additive base analysis model using all fish (n=7,392), fork length (mm) and fractional ages. Linf= 407 mm FL, K= 0.3104, t0= =0.78.

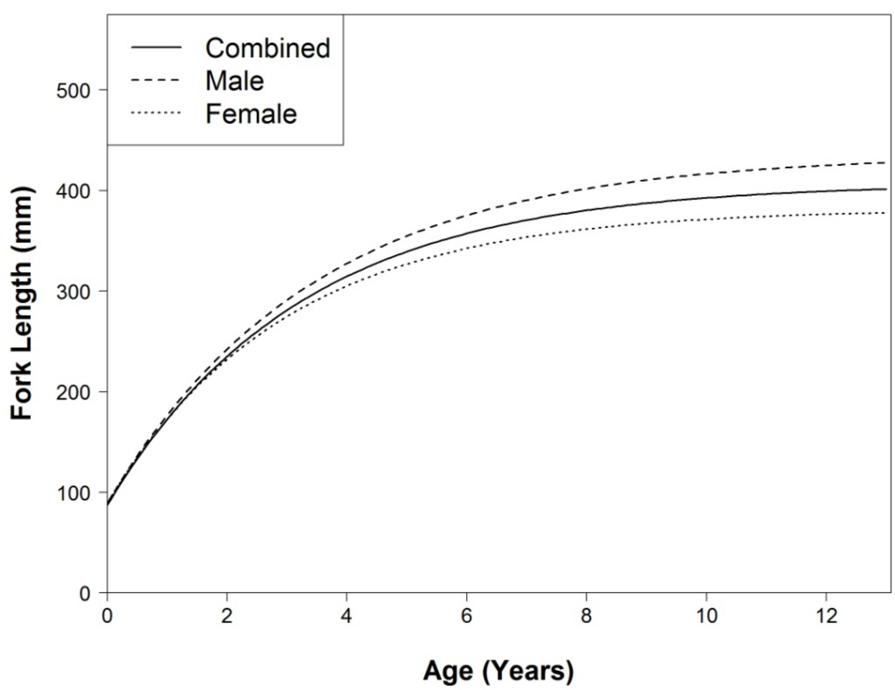
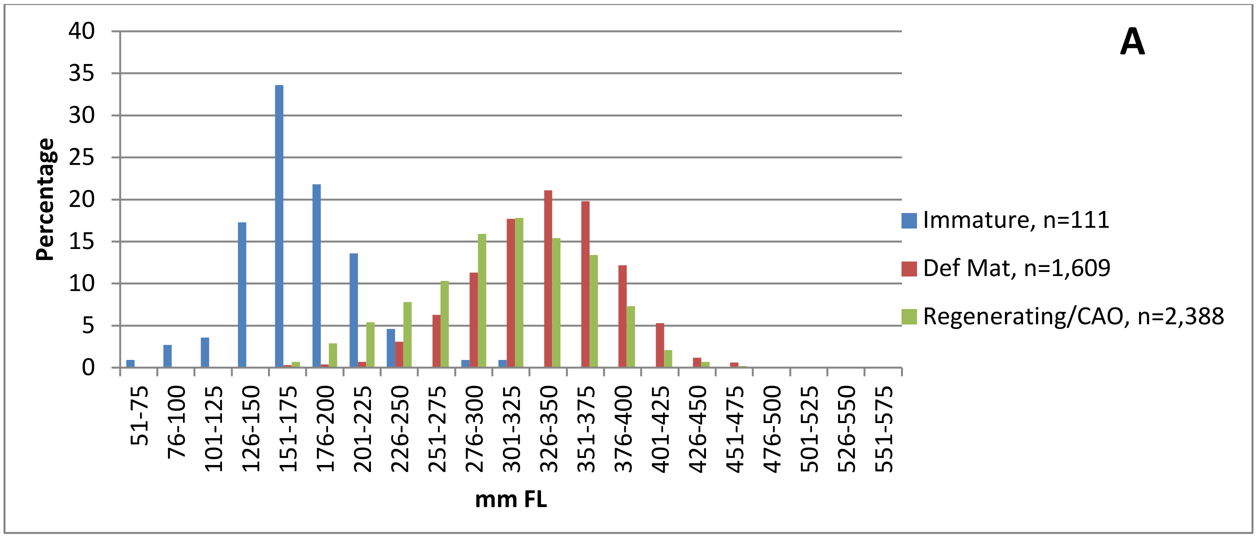


Figure 4. Non-linear regression lines of the Von Bertalanffy growth model analyses of all gray triggerfish combined, males, and females fork length (mm) and fractional age data.



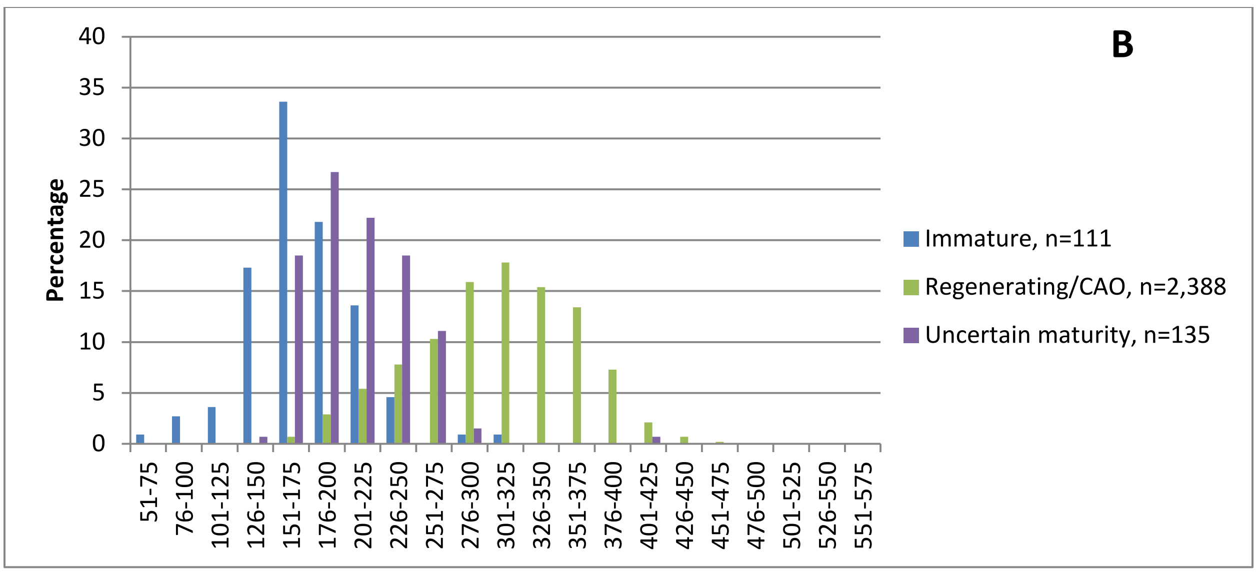
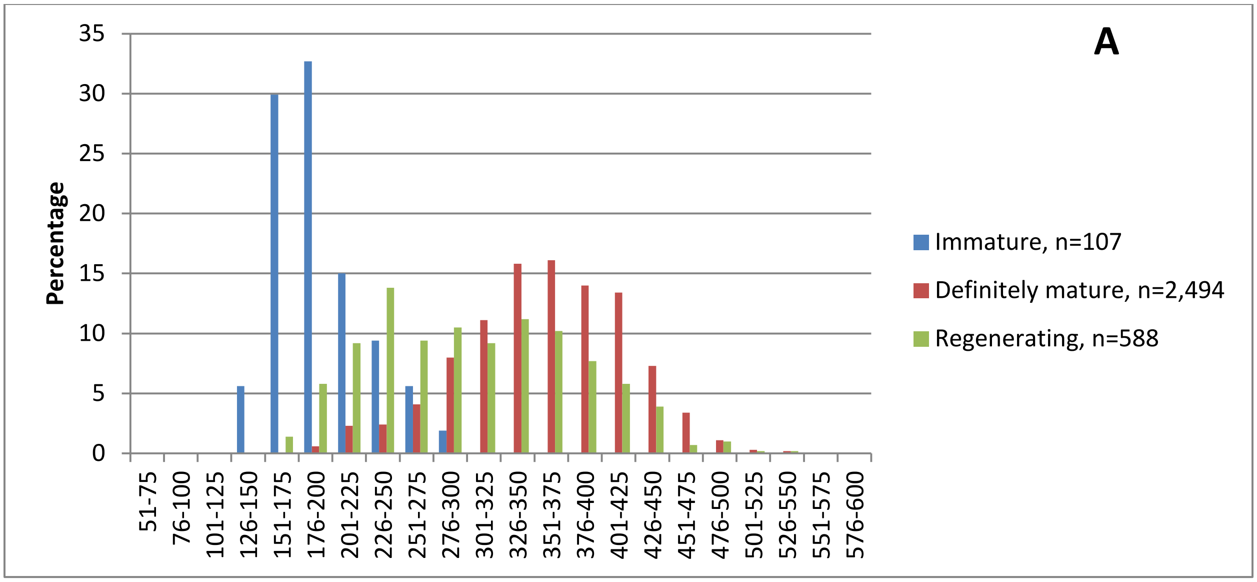


Figure 5. A. A comparison of female gray triggerfish length-frequency histograms specimens that were categorized as immature, definitely mature (Def Mat), or resting. Definitely mature specimens were developing, spawning capable, or regressing. B. Female gray triggerfish histological staging of immature, regenerating and uncertain maturity. Both graphs provide data from all years and all gears. CAO= cortical alveolar oocytes, n= number of fish.



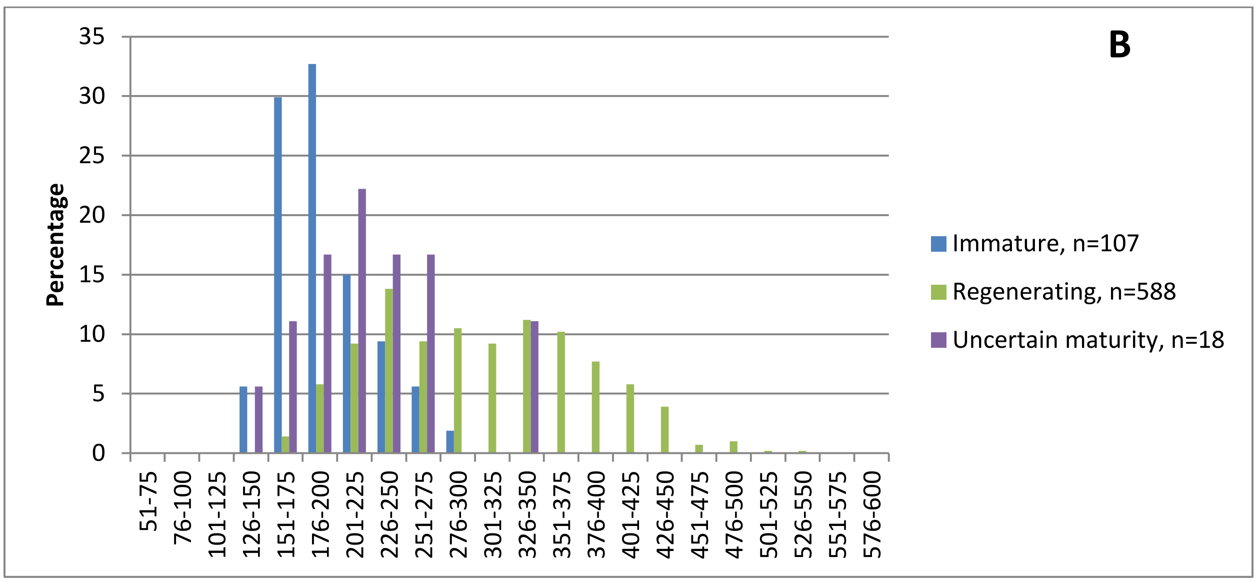


Figure 6. A. A comparison of male gray triggerfish length-frequency histograms specimens that were categorized as immature, definitely mature, or resting. Definitely mature specimens were developing, spawning capable, or regressing. B. Male gray triggerfish histological staging of immature, regenerating and uncertain maturity. Both graphs provide data from all years and all gears. See text and SEDAR32 DW03 for details of maturity states. N= numbers of fish.

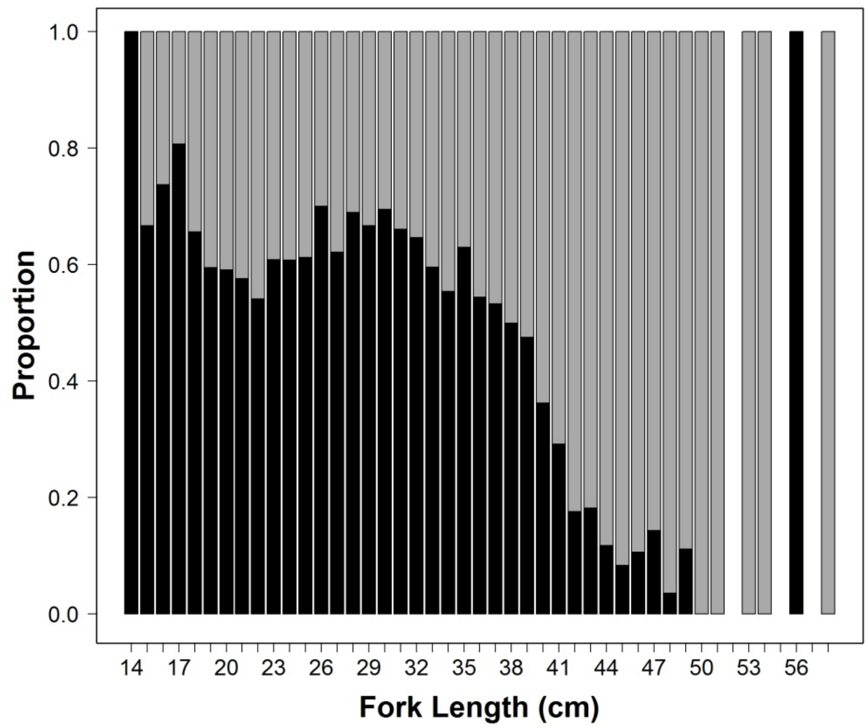


Figure 7. Sex ratio based on fork length. Black bars represent females (total n=4283), gray bars represent males (total n= 3149).

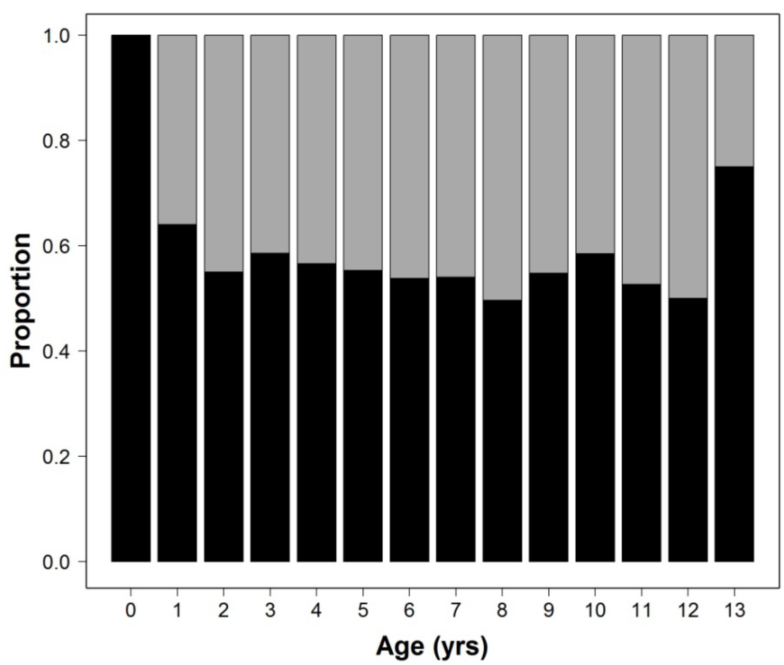


Figure 8. Gray triggerfish age based sex ratio. Female total n= 3892, Male total n= 3118.

ADDENDUM

Marine Resources Monitoring, Assessment and Prediction Program: Report on South Atlantic Gray Triggerfish, *Balistes capriscus*, for the SEDAR 32 Data Workshop.

SEDAR 32-DW05
MARMAP Technical Report 2013-021
Updated February 2013

(vrs.1a)

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Introduction

Gray triggerfish (*Balistes capriscus*) is a marine species in the family Balistidae that occurs in the tropical and temperate zones across the entire Atlantic Ocean, including the Mediterranean Sea (Bernardes 2002, Robins and Ray 1986). Gray triggerfish occur in coastal waters of the western Atlantic from Nova Scotia (Canada) to Argentina, including the Gulf of Mexico and off Bermuda (Bernardes 2002, Robins and Ray 1986). Throughout this distribution gray triggerfish generally are found at depths of 0-100 m (Harmelin-Vivien and Quéro 1990). In the Gulf of Mexico, they are found commonly at depths between 12 and 42 m among reefs and hard bottom habitat (Harper and McClellan 1997).

Gray triggerfish are iteroparous gonochorists, building nests and exhibiting bi-parental care (Mackican and Szedlemayer 2007). Early life stages include demersal eggs and pelagic larvae (Richards and Lindeman 1987). Eggs may not fully hydrate or exhibit the degree of yolk fusion observed in pelagic eggs (Moore 2001). Postovulatory follicles (POFs) are rare in collections possibly due to reduced feeding by spawning females, thereby reducing the chances of females foraging, accepting bait and interacting with collection gear at this phase of the reproductive cycle (Moore 2001). It is unknown if fecundity is determinate or indeterminate. Thus, we know little about female reproductive potential, spawning frequency, and overall ovarian organization.

Male gray triggerfish have separate, small, oval-shaped testes that lie close together on the ventral side of the swim bladder. The common spermatic duct is lined with columnar secretory epithelial cells and surrounded by an accessory gland that may function to secrete substances that maintain spermatozoa while they are stored. Spermatic ducts act as a storage system for spermatozoa before release; therefore, both the testes and the spermatic duct/accessory gland complex are needed to accurately assess reproductive condition. A sample from the testes or duct/gland alone is usually only useful to assess sexual maturity (i.e., juveniles vs. adult).

Previous research on the age and growth of gray triggerfish has been derived predominately from fish outside the jurisdiction of the South Atlantic Fisheries Management Council (SAFMC). Peer-reviewed and unpublished studies in other regions, using the first dorsal spine as the aging structure, include the southern coast of Africa (Caveriviere et al. 1981, Ofori-Danson 1989, Aggrey-Fynn 2009), Brazil (Bernardes 2002), and the Gulf of Mexico (Johnson and Saloman 1984, Wilson et al. 1995, Hood and Johnson 1997, Ingram 2001, Fioramonti 2012). Along the US South Atlantic, only two of these have focused on the age and growth of gray triggerfish in coastal waters (Escorriola 1991, Moore 2001). Moore (2001) found that gray triggerfish collected among reefs and hard bottom habitat from Cape Fear, North Carolina to Cape Canaveral, Florida ranged in age from 0 to 10 years old, with a maximum observed fork length (FL) of 560 mm. Moore (2001) also found that males were significantly larger than females. To our knowledge, all previous studies conducted on the age and growth of gray triggerfish utilized the first dorsal spine as the primary aging structure. The spine is used rather than the otoliths due to the extremely small size and irregular shape of gray triggerfish otoliths. This makes routine extraction and examination of otoliths in this species difficult and time consuming compared to other species. Currently, no published documentation exists of comparisons among potential aging structures (spines, otoliths, vertebrae, etc.) in gray triggerfish.

Gray triggerfish from the US South Atlantic are undergoing an inaugural benchmark stock assessment through the SouthEast Data, Assessment, and Review (SEDAR) process in 2013 (SEDAR 32). This assessment will include data through 2011.

This report describes the data collected by the Marine Resources Monitoring, Assessment, and Prediction (MARMAP), Southeast Atlantic Monitoring, Assessment, and Prediction (SEAMAP-SA) and Southeast Fishery Independent Survey (SEFIS) programs (for details of these programs see below and Ballenger et al. 2011).

Methods

Spines and gonadal tissues were taken from gray triggerfish specimens collected from coastal and offshore waters between Cape Hatteras, North Carolina, and St. Lucie Inlet, Florida, between 1973-2011 (N=8,607). The vast majority of specimens were collected during standard sampling by the MARMAP program (fishery-independent, Project ID: P05, P55, & Q26) and using chevron traps (gear code 324), but over the years other gears collected gray triggerfish such as Florida traps (gear code 074), blackfish traps (gear code 053), mini-Antillean "S" traps (gear code 041), 3/4 scale Yankee trawl (gear code 022), snapper/bandit reel (gear code 043) hook and line (gear code 014), spear gun (gear code 065), Experimental trap (gear code 073), and Lionfish trap (gear code 540) (Collins 1990, Harris and McGovern 1997, Harris et al. 2004, MARMAP 2009). MARMAP/SEAMAP-SA provided 62 specimens between 2009-2011. SEFIS also provided samples using chevron traps and hook and line since 2010. Thirty eight gray triggerfish were collected during standard sampling by the SEAMAP program (fishery-independent, Project P94), using Mongoose-type Falcon trawl (gear code 233). Gray triggerfish specimens were also obtained from commercial catches (fishery-dependent, Project ID: P50) using hook and line (gear code 014), dip net (gear code 019), snapper/bandit reel (gear code 043), and chevron trap (gear code 324).

Workshops were held in Charleston SC (September 2011) and NOAA Fisheries SEFSC-Beaufort Laboratory (October 2012) in preparation for SEDAR 32. The goals of the workshops were to (1) compare sample preparation, reading methods and data analysis of the first dorsal spine of gray triggerfish, with an emphasis on addressing difficulties and issues previously encountered by Gulf of Mexico and Atlantic labs, and (2) compare reproductive histological assessments and finalize methodology and analyses (see SEDAR DW-03 for results).

After collection, catches were sorted by species and processed following standard protocols (see details in MARMAP 2009). Whole gray triggerfish were weighed to the nearest gram (g) and total length (TL), fork length (FL), and standard length (SL) were measured to the nearest mm. Note that fork length was used in all length-based analyses in this report based on the SEDAR 32 Scoping Conference Call. Spines were removed from all fish and stored dry prior to processing. Samples of gonad tissues were removed and stored in 11% seawater buffered formalin until later processing.

Age

Spine sections were processed using standard methods as discussed and agreed upon by various collaborating fish aging labs that are providing age data to SEDAR 32 (SEDAR32-DW03).
MARMAP utilized transverse sections of the dorsal spine immediately distal to the condyle groove for age determination. Spines were cleaned to a degree that surplus skin and muscle tissue was removed prior to sectioning. An Isomet low-speed saw was used to cut 0.4-0.7 mm thick sections from gray triggerfish spines. The workshop concluded that the increments as identified by the workshop participants can be considered annuli, and can be used to determine the age of gray triggerfish.
At SC-DNR, spine sections were examined independently by two readers and re-examined jointly when differences in age estimation occurred. Aging was done without knowledge of specimen length or date of capture. If disagreement persisted, the specimen was eliminated from age analyses. In addition, we recorded quality and edge type (Table 1).

Based on evidence for a June-July spawning peak in females (Figure 1), the workshop recommended the use of the following criteria to convert increment counts to annual ages: any fish captured prior to July 1st with an edge type of 3 or 4 were assigned a calendar age of increment count plus one, otherwise calendar age equals increment count. Calendar ages were used in analyses of sex ratio, male and female age at maturity, age compositions, and spawning periodicity. Fractional ages were assigned based on calendar age and adjusted for date of capture assuming a July 1 birthday. Fractional age was used in growth models, age vs. depth, and fecundity analyses.

<u>Reproduction</u>
Following capture and dissection, the posterior portion of the gonads were fixed for 7–14 d in an 11% seawater–formalin solution buffered with marble chips and transferred to 50% isopropanol for an additional 7–14 d. Male gray triggerfish are unique in that both testes and the spermatic duct/accessory gland must be collected for complete analysis. For this reason, two different sections of the spermatic duct/accessory gland were taken along with a sample of the testes to ensure accurate staging. Reproductive tissue was processed in an automated and self-enclosed tissue processor and blocked in paraffin. Three transverse sections (6–8 µm thick) were cut from each sample with a rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counterstained with eosin-y. Sections were viewed under a compound microscope at 20-400X magnification, and sex and reproductive class were determined without knowledge of capture date, specimen length, or specimen age. Descriptive criteria for reproductive classes with the inclusion of subclasses for male staging was outlined and recommended during the gray triggerfish workshops (Table 2 and Table 3). Three readers independently determined sex and reproductive state using histological criteria (Moore 2001, Wyanski 2006, and Brown-Peterson et al. 2011). When assignments differed, the readers re-examined the section simultaneously to determine reproductive state. Females were considered to be in spawning condition if they possessed oocytes undergoing maturation (i.e., fusing of yolk globules, germinal vesicle migration and breakdown) or postovulatory follicles (POFs).

<u>Analyses</u>
All analyses were done using “R”. In some instances the data set was subdivided based on depth, latitudinal and temporal state. The following criteria was used during for these analyses:

Depth:

	Inshore:	Samp_depth < 30m;
	Offshore:	Samp_depth >= 30m

Latitude:

	South:	Latitude < 32 degrees;
	North:	Latitude >= 32 degrees

Period:

	Early:	Year<1990;
	Mid :	1989 < Year < 2000;
	Late:	Year>1999

Lengths were recorded in mm and weight in grams. Length/length and length/weight conversions were log transformed and analyzed using linear regression analyses. As we have no gutted weight data available, no weight/weight analyses were done. Sex ratio data were analyzed using a Chi-square goodness of fit test to determine if these ratios differed among size classes from an expected 1:1 (Zar 1984). R Statistical Software was used to estimate length at 50% maturity (L50) and age at 50% maturity

(A50). Workshop participants also recommended using gonad weight versus FL and whole fish weight as a proxy for a fecundity estimate. The R^2 values are adjusted for degrees of freedom.

<u>Results</u>

Gray triggerfish analyzed for this report were captured between latitude 27.23^0 and 35.10^0 and at a depth range of 0 to 93 meters. Specimens ranged in fork length from 75 to 578 mm and ranged in weight from 11 to 5,000 g. Ages ranged from 0 to 13.

<u>Length/length and length/weight conversions.</u>

Linear regression analyses indicated that there were no significant differences in the slopes of various length/length regressions between males and females (Table 4) or in the slopes of length (mm) versus weight (g) between males and females (Table 5, Figure 2). In both cases, assuming equal slopes, there was a significant difference in intercepts between males and females. However, these differences were a result of a large data set and have no biological relevance. Our recommendation is to use conversion equations for males and females combined (Table 4).

<u>Length-at-Age</u>

Length-age data from males, females and all data was fitted to the von Bertalanffy growth model (FL = Linf*exp(-k*(age-t_0) to generate estimates of growth parameters for gray triggerfish (Table 6, Figure 3 and 4). The results show differences in growth rate between the sexes and our recommendation is to use sex-specific growth von Bertalanffy parameters.

<u>Reproduction</u>

There was a high degree of overlap in the length distributions of definitely mature and regenerating gray triggerfish and modest overlap in the lengths of immature and all mature individuals, indicating that individuals were correctly assigned to the immature and regenerating classes (Figures 5 & 6).

The results of all modeling indicate that gray triggerfish mature before they reach one year of age. Age based maturity analyses were done using calendar age. Size (1 cm size bins) and age (by year) at maturity was based on a Logit model, as it provided the best fit. Female size and age at maturity yielded an A50 = 0.71 yr and L50 = 174 mm (Table 7, 8, and 9). Male length and age at maturity yielded A50 = 0.88 yr and L50 = 181mm (Table 10, 11, and 12).

The overall sex ratio of 1.208 was significantly different form a 1:1 ratio, with the proportion of females being greater than that for males (Table 13). However, analyses also indicated that female gray triggerfish were more abundant than males at smaller sizes, while male gray triggerfish were significantly more abundant at sizes $\geq$ 400mm FL (Table 14, Table 15, Figure 7, Figure 8).

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Table 1. Spine edge type and quality.

EDGE TYPE

Code	Description	
1	Opaque zone on the edge.	
2	Narrow translucent zone on edge	Width less than about 30% of previous increment
3	Medium translucent zone on edge	Width about 30-60% of previous increment
4	Wide translucent zone on edge	Width more than about 60% of previous increment

READABILITY

Code		Description and analysis consequence
A	Unreadable	Omit otolith from analysis
B	Very difficult to read	Age estimate between readers are expected to be >2 year for young, and > 4 yrs for old fish (>10 yrs) Agreement on age may be difficult to reach, in which case otoliths should be classified as A and omitted from the analysis.
C	Fair readability	Age estimates between readers should be within 2 year in young, and within 4 years in old fish (>10 yrs). Agreement after second reading is expected after some discussion.
D	Good readability	Age estimates between readers should be within 1 year for young, to 2 years in old fish (> 10 years). Agreement after second reading is expected without much discussion.
E	Excellent readability	Age estimates between readers should be the same.

Table 2: Histological interpretation of female gray triggerfish. Most descriptors based on Moore (2001), Wyanski (2006) and Brown-Peterson et al. (2011).

Maturity Class	**Description**
Uncertain Maturity (Class 0)	Inactive ovaries, primary growth oocytes only; unable to assess maturity
Immature (Class 1)	Primary growth oocytes 20-60 micron diameter (Moore 2001); no evidence of atresia. In comparison to regenerating female, transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, oogonia abundant along margin of lamellae, ovarian wall is thinner
Cortical alveolar oocytes (Class E)	Early Developing; Previtellogenic; cortical alveolar oocytes 140-200 micron diameter
Yolked oocytes (Class F)	Vitellogenic; Most advanced oocytes in yolk-granule or yolk-globule stage; oocyte 170-400 micron diameter
Migratory nucleus oocytes (Class G)	Oocyte maturation; partial coalescence of yolk globules possible; Oocytes 385-500 micron diameter
Postovulatory follicles (POFs): early (Class B), intermediate (Class C), late (Class D)	Vitellogenic oocytes and POFs; Evidence of recent spawn; note that beta-stage atresia cannot always be distinguished from medium to old postovulatory follicles (Hunter and Macewicz 1985)
Regressing (Class 4)	>50% of yolked oocytes undergoing alpha or beta stage of atresia
Regenerating (Class 5)	Primary growth oocytes > 60 micron 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
Mature specimen (Class 8)	Mature, but postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive class
Unknown (Class 9)	Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state

Table 3: Histological interpretation of male gray triggerfish. Most descriptors based on Moore (2001), Wyanski (2006) and Brown-Peterson et al. (2011) with the inclusion of sub-classes.

Maturity Class	Sub-Class	Description
Uncertain Maturity (Class 0)		Inactive testes; unable to assess maturity
Immature (Class 1)		Small transverse section compared to regenerating male; little or no spermatocyte development
Developing (Class 2)		Limited spermatogenesis in testes; elongation of lobules and some accumulation of spermatozoa (SZ) in testes BUT no accumulation in lobules, efferent ducts (within testes), and spermatic ducts
Spawning Capable (3 sub-classes)	Early Spawning Capable (Subclass ESC)	Spermatozoa evident in ducts; amount of spermatogenesis in testes ranges from limited to extensive; in ducts, greater area of structural tissue compared to sinuses
	Storage (Subclass H)	Spermatozoa storage within expanding ducts; >50% of area of sinuses densely packed with spermatozoa; amount of spermatogenesis in testes ranges from limited to extensive
	Recent Spawn (Subclass 7)	Large, expanded ducts not as densely packed with spermatozoa; area of sinuses greater than that of structural tissue; usually has empty lobules toward center of testes
Regressing (Class 4)		Limited spermatogenesis in testes; shrinking ducts/lobules with residual spermatozoa present; overall number of ducts containing spermatozoa also small; increase of connective tissue in testes, proliferating from center; may have enlarged cells lining sinuses
Regenerating (Class 5)		Larger transverse section compared to immature male; very limited or no spermatogenesis in testes; little or no residual spermatozoa in ducts
Mature Specimen (Class 8)		Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive class
Unknown (Class 9)		Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state

Table 4. Gray Triggerfish length versus length relationships. TL=total length, FL=fork length, SL=stndard length. All lengths are in mm. n=number of combinations available and used for analyses. R2 is adjusted for degrees of freedom.

	equation	n	a	b	R2
All	TL=a+b*FL	8543	-17.347	1.208	0.9677
	TL=a+b*SL	8541	2.059	1.381	0.9530
	FL=a+b*SL	8591	16.173	1.144	0.9833
Males	TL=a+b*FL	3621	-19.616	1.215	0.9657
	TL=a+b*SL	3618	1.504	1.388	0.9514
	FL=a+b*SL	3652	17.722	1.141	0.9835
Females	TL=a+b*FL	4376	-15.005	1.200	0.9633
	TL=a+b*SL	4375	4.832	1.369	0.9451
	FL=a+b*SL	4391	16.927	1.139	0.9791

Table 5. Length (mm) versus weight (g) relationships. FISH_WT=fish wet weight in grams, TL=total length, FL=fork length, SL=standard length. All lengths are in mm. n=number of combinations available and used for analyses. R2 is adjusted for degrees of freedom.

	equation	n	a	b	R2
All	Log (WT) = log a + bTL	8522	-9.570	2.742	0.9614
	Log (WT) = log a + bFL	8571	-10.359	2.946	0.9852
	Log (WT) = log a + bSL	8571	-9.046	2.810	0.9753
Males	Log (WT) = log a + bTL	3611	-9.592	2.744	0.9587
	Log (WT) = log a + bFL	3646	-10.463	2.962	0.9861
	Log (WT) = log a + bSL	3644	-9.100	2.818	0.9758
Females	Log (WT) = log a + bTL	4366	-9.613	2.751	0.9575
	Log (WT) = log a + bFL	4381	-10.387	2.953	0.9826
	Log (WT) = log a + bSL	4381	-0.9033	2.808	0.9706

Table 6. Estimates of Von Bertalanffy growth parameters base on non-linear regression analysis using available total length (mm) and age (fractional age) data. N=number of aged fish used in analysis. Linf= asymptotic FL, SE= standard error, K= Von Bertalanffy growth parameter. T0= VonBertanffy parameter for age at theoretical length=0.

	n	Linf	SE	k	SE	t0	SE
All Fish	7,391	408	3.31	0.311	0.0115	-0.75	0.082
Males	3,208	437	5.87	0.290	0.0166	-0.79	0.128
Females	3,980	382	3.46	0.340	0.0153	-0.75	0.102

Table 7. Results of various regression model analyses for age and length at maturity for female gray triggerfish. Data for all years and all gears were combined. Age is expressed in fractional age and length is fork length in mm. N=number of fish used in analyses, a/lamda= coefficient, b/k= coefficient, A50= age at 50% maturity, L50=length at 50% maturity. AIC=Akaike's Information Criterion. Parameters in bold represent the best fit models.

	Model	N	a/lamda	SE	b/k	SE	A50/L50	AIC
Age	**Logistic Logit**	**3860**	**-0.984**	**0.283**	**1.393**	**0.108**	**0.710**	**609.09**
	Logistic Probit	3860	-0.171	0.152	0.617	0.051	0.277	617.96
	Logistic - clog-log	3860	0.026	0.114	0.352	0.034	-0.074	638.44
	Logistic - Cauchy	3860	-5.051	0.725	5.248	0.620	0.962	681.15
	Gompertz	3860	2.179	0.301	1.243	0.056	0.95	-4176.84
Length	**Logistic Logit**	**4251**	**-10.252**	**0.872**	**0.059**	**0.004**	**174**	**387.95**
	Logistic Probit	4251	-4.503	0.398	0.027	0.002	169	411.82
	Logistic - clog-log	4251	-2.409	0.270	0.015	0.001	166	471.88
	Logistic - Cauchy	4251	-41.730	5.569	0.241	0.032	173	462.59

Table 8. Female fork length (mm) at maturity using Logistic-Logit. % Mature= Percent Mature, 0.5= length (fork length in mm) at maturity, Prop. Mat= projected proportion mature using Logit model.

Length	Immature	Mature	Total	% Mature	0.5	Prop. Mat
8	2	0	2	0.000	174	0.004
9	0	0	0	NA	174	0.007
10	3	0	3	0.000	174	0.013
11	2	0	2	0.000	174	0.023
12	1	0	1	0.000	174	0.041
13	3	0	3	0.000	174	0.071
14	8	1	9	0.111	174	0.121
15	9	1	10	0.100	174	0.199
16	17	3	20	0.150	174	0.310
17	17	15	32	0.469	174	0.448
18	9	22	31	0.710	174	0.594
19	9	30	39	0.769	174	0.726
20	8	46	54	0.852	174	0.827
21	5	45	50	0.900	174	0.896
22	9	73	82	0.890	174	0.940
23	1	66	67	0.985	174	0.966
24	4	110	114	0.965	174	0.981
25	0	104	104	1.000	174	0.989
26	0	155	155	1.000	174	0.994
27	0	123	123	1.000	174	0.997
28	0	225	225	1.000	174	0.998
29	0	203	203	1.000	174	0.999
30	1	327	328	0.997	174	0.999
31	0	273	273	1.000	174	1.000
32	0	340	340	1.000	174	1.000
33	0	264	264	1.000	174	1.000
34	0	322	322	1.000	174	1.000
35	0	265	265	1.000	174	1.000
36	0	317	317	1.000	174	1.000
37	0	203	203	1.000	174	1.000
38	0	206	206	1.000	174	1.000
39	1	134	135	0.993	174	1.000
40	0	116	116	1.000	174	1.000
41	0	63	63	1.000	174	1.000
42	0	38	38	1.000	174	1.000
43	0	22	22	1.000	174	1.000
44	0	12	12	1.000	174	1.000
45	0	6	6	1.000	174	1.000
46	0	5	5	1.000	174	1.000
47	0	3	3	1.000	174	1.000
48	0	1	1	1.000	174	1.000

49	0	1	1	1.000	174	1.000
50	0	0	0	NA	174	1.000
51	0	0	0	NA	174	1.000
52	0	0	0	NA	174	1.000
53	0	0	0	NA	174	1.000
54	0	0	0	NA	174	1.000
55	0	0	0	NA	174	1.000
56	0	1	1	1.000	174	1.000

Table 9. Female age at maturity using Logistic-Logit. Female age at maturity using Logistic-Logit. % Mature= Percent Mature, 0.5= age at maturity, Prop. Mat= projected proportion mature using Logit model. Calendar age was used in these analyses.

Age	Immature	Mature	Total	% Mature	0.5	Prop Mat
0	4	0	4	0.000	0.69	0.275
1	23	39	62	0.629	0.69	0.606
2	34	212	246	0.862	0.69	0.862
3	23	564	587	0.961	0.69	0.962
4	9	846	855	0.989	0.69	0.990
5	0	821	821	1.000	0.69	0.998
6	0	612	612	1.000	0.69	0.999
7	0	363	363	1.000	0.69	1.000
8	1	177	178	0.994	0.69	1.000
9	0	85	85	1.000	0.69	1.000
10	0	31	31	1.000	0.69	1.000
11	0	10	10	1.000	0.69	1.000
12	0	3	3	1.000	0.69	1.000
13	0	3	3	1.000	0.69	1.000

Table 10. Results of various regression model analyses for age and length at maturity for male gray triggerfish. Data for all years and all gears were combined. Age is expressed in fractional age and length is fork length in mm. n=number of fish used in analyses, a/lamda= coefficient, b/k= coefficient , A50= age at 50% maturity, L50=length at 50% maturity. AIC=Akaike's Information Criterion. Parameters in bold represent the best fit models.

	Model	N	a/lamda	SE	b/k	SE	A50/L50	AIC
Age	**Logistic Logit**	**3163**	**-1.261**	**0.295**	**1.428**	**0.115**	**0.88**	**559.39**
	Logistic Probit	3163	-0.331	0.158	0.641	0.054	0.51	568.39
	Logistic - clog-log	3163	-0.226	0.126	0.413	0.038	0.55	580.48
	Logistic - Cauchy	3163	-5.798	0.761	5.266	0.641	1.10	602.75
	Gompertz	3163	5.617	0.920	1.540	0.074	0.96	-3044.04
Length	**Logistic Logit**	**3602**	**-9.019**	**0.825**	**0.050**	**0.004**	**181**	**434.99**
	Logistic Probit	3602	-4.402	0.426	0.025	0.002	178	440.52
	Logistic - clog-log	3602	-3.438	0.375	0.018	0.002	189	454.06
	Logistic - Cauchy	3602	-30.938	3.898	0.168	0.021	184	509.53

Table 11. Male fork length (mm) at maturity using Logistic Logit. % Mature= Percent Mature, 0.5= length (fork length in mm) at maturity, Prop. Mat= projected proportion mature using Logit model.

					Logistic Logit	
Length	**Immature**	**Mature**	**Total**	**% Mature**	**0.5**	**Prop. Mat**
13	1	0	1	0.000	181	0.074
14	4	0	4	0.000	181	0.116
15	6	0	6	0.000	181	0.178
16	8	2	10	0.200	181	0.263
17	16	6	22	0.273	181	0.370
18	14	18	32	0.563	181	0.492
19	17	24	41	0.585	181	0.615
20	12	39	51	0.765	181	0.724
21	5	36	41	0.878	181	0.812
22	6	65	71	0.915	181	0.877
23	2	42	44	0.955	181	0.922
24	5	80	85	0.941	181	0.951
25	5	66	71	0.930	181	0.970
26	1	65	66	0.985	181	0.981
27	2	79	81	0.975	181	0.989
28	2	99	101	0.980	181	0.993
29	1	101	102	0.990	181	0.996
30	0	144	144	1.000	181	0.997
31	0	140	140	1.000	181	0.998
32	0	187	187	1.000	181	0.999
33	0	177	177	1.000	181	0.999
34	0	259	259	1.000	181	1.000
35	0	156	156	1.000	181	1.000
36	0	266	266	1.000	181	1.000
37	0	177	177	1.000	181	1.000
38	0	207	207	1.000	181	1.000
39	0	148	148	1.000	181	1.000
40	0	205	205	1.000	181	1.000
41	0	152	152	1.000	181	1.000
42	0	178	178	1.000	181	1.000
43	0	99	99	1.000	181	1.000
44	0	90	90	1.000	181	1.000
45	0	66	66	1.000	181	1.000
46	0	42	42	1.000	181	1.000
47	0	24	24	1.000	181	1.000
48	0	26	26	1.000	181	1.000
49	0	8	8	1.000	181	1.000
50	0	9	9	1.000	181	1.000
51	0	7	7	1.000	181	1.000
52	0	0	0	NA	181	1.000
53	0	3	3	1.000	181	1.000
54	0	2	2	1.000	181	1.000
55	0	0	0	NA	181	1.000
56	0	0	0	NA	181	1.000
57	0	0	0	NA	181	1.000
58	0	1	1	1.000	181	1.000

Table 12. Male age at maturity using Logistic Logit. % Mature= Percent Mature, 0.5= age at maturity, Prop. Mat= projected proportion mature using Logit model. Calendar age was used in these analyses.

Age	Immature	Mature	Total	% Mature	0.5	Prop. Mat
0	1	0	1	0.000	0.83	0.238
1	29	27	56	0.482	0.83	0.559
2	35	200	235	0.851	0.83	0.837
3	14	410	424	0.967	0.83	0.954
4	10	651	661	0.985	0.83	0.988
5	3	665	668	0.996	0.83	0.997
6	1	527	528	0.998	0.83	0.999
7	0	305	305	1.000	0.83	1.000
8	0	180	180	1.000	0.83	1.000
9	0	71	71	1.000	0.83	1.000
10	0	21	21	1.000	0.83	1.000
11	0	9	9	1.000	0.83	1.000
12	0	3	3	1.000	0.83	1.000
13	0	1	1	1.000	0.83	1.000

Table 13. Gray triggerfish overall sex ratio.

	Ratio: Female:Male	# Male	# Female	Proportion Female	Chi-squared	P-value
Overall	1.208	3550	4287	0.547	69.308	<0.0001

Table 14. Length based sex ratio by one centimeter bins.

Fork Length	Female:Male	# Male	# Female	Proportion	Chi-square	P-value
14	n/a	0	2	1.000	n/a	n/a
15	2.000	1	2	0.667	n/a	n/a
16	2.800	5	14	0.737	4.263	0.39
17	4.167	6	25	0.806	11.645	0.001
18	1.909	22	42	0.656	6.250	0.012
19	1.467	30	44	0.595	2.649	0.104
20	1.444	45	65	0.591	3.636	0.057
21	1.357	42	57	0.576	2.273	0.132
22	1.179	67	79	0.541	0.986	0.321
23	1.553	47	73	0.608	5.633	0.018
24	1.550	80	124	0.608	9.490	0.002
25	1.580	69	109	0.612	8.989	0.003
26	2.333	69	161	0.700	36.800	<0.0001
27	1.638	80	131	0.621	12.327	<0.0001
28	2.216	102	226	0.689	46.878	<0.0001
29	2.000	102	204	0.667	34.000	<0.0001
30	2.278	144	328	0.695	71.729	<0.0001
31	1.943	140	272	0.660	42.291	<0.0001
32	1.824	187	341	0.646	44.917	<0.0001
33	1.470	181	266	0.595	16.163	<0.0001
34	1.242	260	323	0.554	6.808	0.009
35	1.699	156	265	0.629	28.221	<0.0001
36	1.192	266	317	0.544	4.461	0.035
37	1.140	178	203	0.533	1.640	0.2
38	0.995	207	206	0.499	0.002	0.961
39	0.905	148	134	0.475	0.695	0.405
40	0.568	206	117	0.362	24.523	<0.0001
41	0.412	153	63	0.292	37.500	<0.0001
42	0.213	178	38	0.176	90.741	<0.0001
43	0.222	99	22	0.182	49.000	<0.0001
44	0.133	90	12	0.118	59.647	<0.0001
45	0.091	66	6	0.083	50.000	<0.0001
46	0.119	42	5	0.106	29.128	<0.0001
47	0.167	24	4	0.143	14.286	<0.0001
48	0.037	27	1	0.036	24.143	<0.0001
49	0.125	8	1	0.111	n/a	n/a
50	0.000	9	0	0.000	n/a	n/a
51	0.000	7	0	0.000	n/a	n/a
53	0.000	3	0	0.000	n/a	n/a
54	0.000	2	0	0.000	n/a	n/a
56	n/a	0	1	1.000	n/a	n/a
58	0.000	1	0	0.000	n/a	n/a

Table 15. Age based sex ratio by one centimeter fork length bins.

	Female:Male	# Male	# Female	Proportion	Chi-square	P-value
0	n/a	0	1	1.0000	n/a	n/a
1	1.778	36	64	0.6400	7.84	0.0051
2	1.223	211	258	0.5501	4.71	0.03
3	1.415	417	590	0.5859	29.721	<0.0001
4	1.305	660	861	0.5661	26.562	<0.0001
5	1.238	669	828	0.5531	16.888	<0.0001
6	1.164	531	618	0.5379	6.588	0.01
7	1.175	308	362	0.5403	4.352	0.037
8	0.983	180	177	0.4958	0.025	0.874
9	1.211	71	86	0.5478	1.433	0.231
10	1.409	22	31	0.5849	1.528	0.216
11	1.111	9	10	0.5263	0.053	0.819
12	1.000	3	3	0.5000	n/a	n/a
13	3.000	1	3	0.7500	n/a	n/a

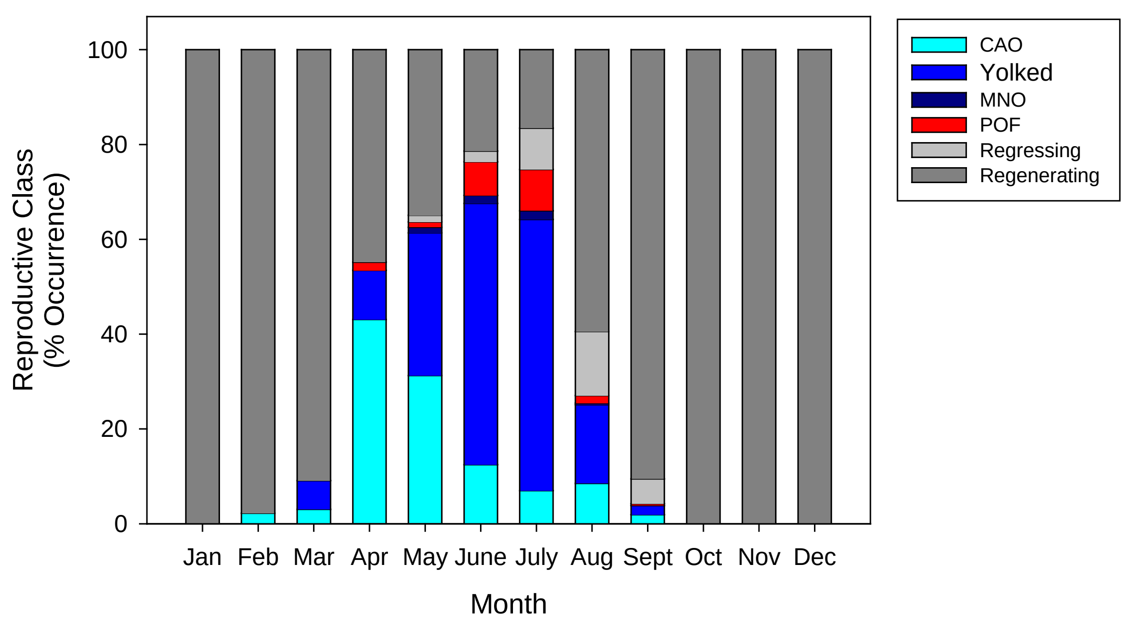

Figure 1. Female gray triggerfish spawning seasonality. CAO= cortical alveolar oocytes, Yolked= Yolked oocytes, MNO= migratory nuclear oocytes, POF= post ovulatory ooctyes.

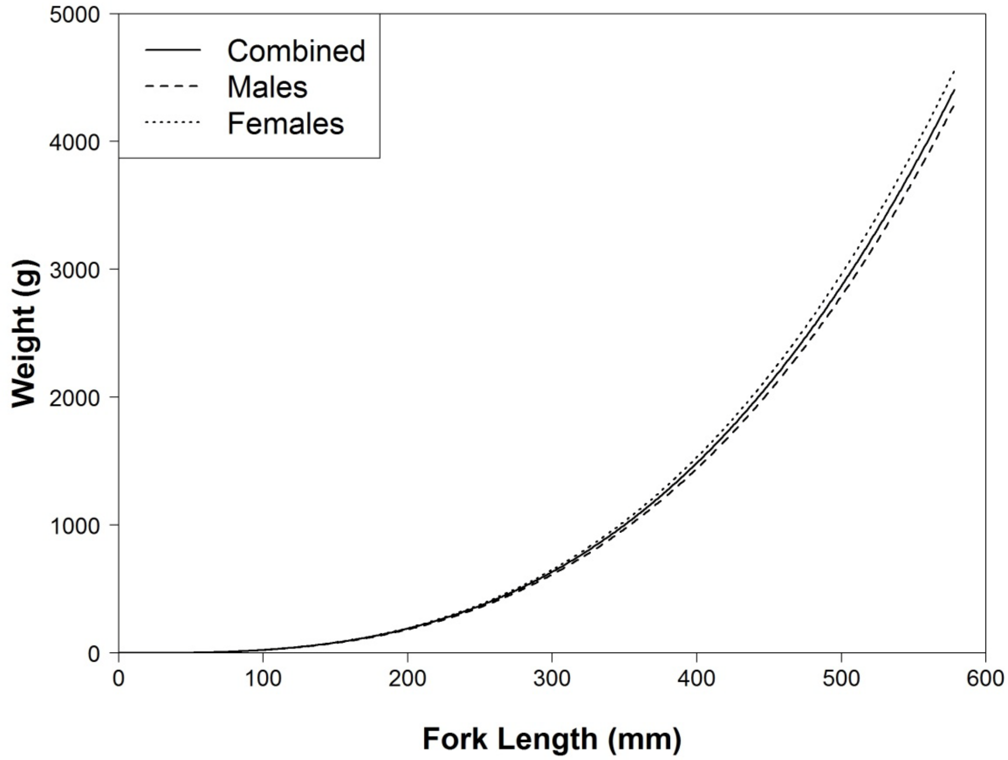

Figure 2. Regression lines of analyses of gray triggerfish fork length (mm) versus whole wet weight (g).

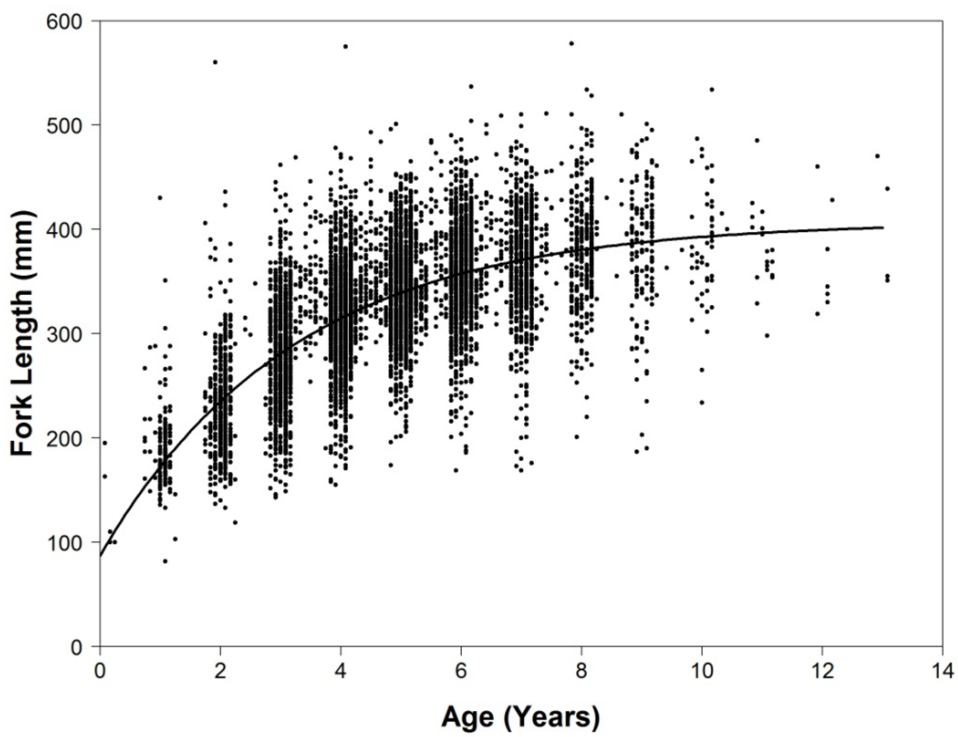

Figure 3. Gray triggerfish Von Bertalanffy additive base analysis model using all fish (n=7,391), fork length (mm) and fractional ages. Linf= 408 mm FL, K= 0.311, t0= =0.75.

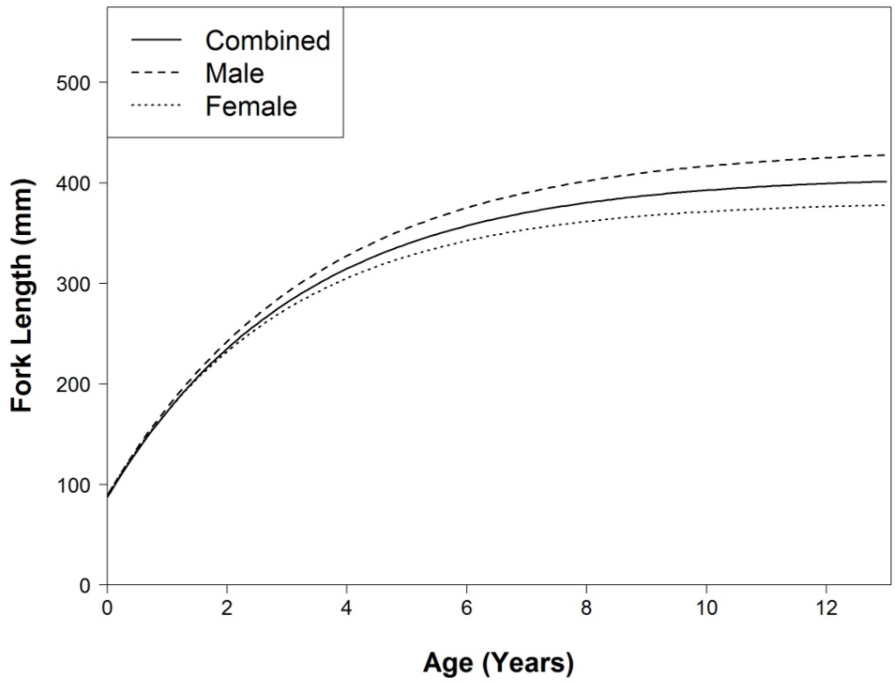

Figure 4. Non-linear regression lines of the Von Bertalanffy growth model analyses of all gray triggerfish combined, males, and females fork length (mm) and fractional age data.

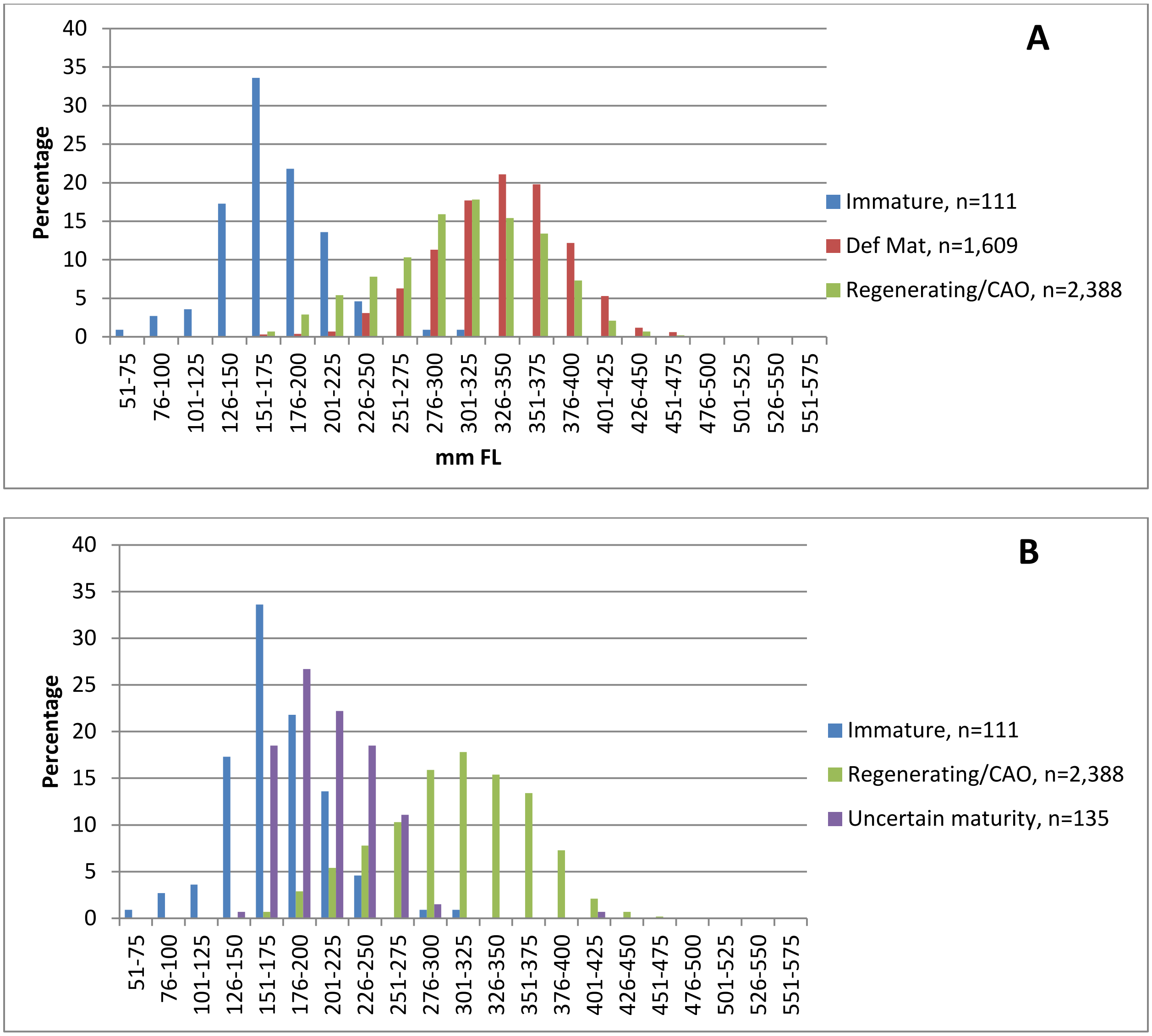

Figure 5. A. A comparison of female gray triggerfish length-frequency histograms specimens that were categorized as immature, definitely mature (Def Mat), or resting. Definitely mature specimens were developing, spawning capable, or regressing. B. Female gray triggerfish histological staging of immature, regenerating and uncertain maturity. Both graphs provide data from all years and all gears. CAO= cortical alveolar oocytes, n= number of fish.

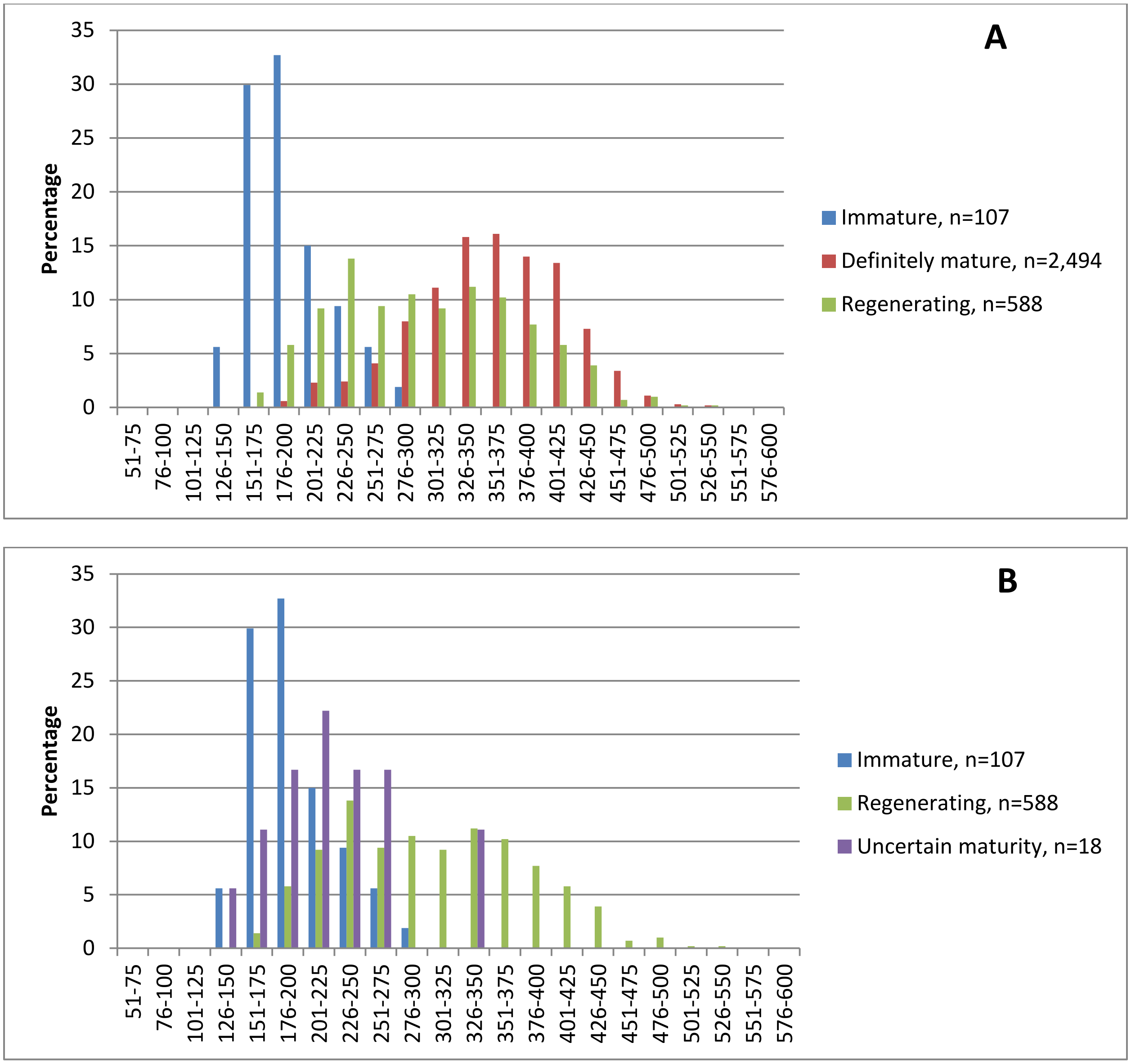

Figure 6. A. A comparison of male gray triggerfish length-frequency histograms specimens that were categorized as immature, definitely mature, or resting. Definitely mature specimens were developing, spawning capable, or regressing. B. Male gray triggerfish histological staging of immature, regenerating and uncertain maturity. Both graphs provide data from all years and all gears. See text and SEDAR32 DW03 for details of maturity states. N= numbers of fish.

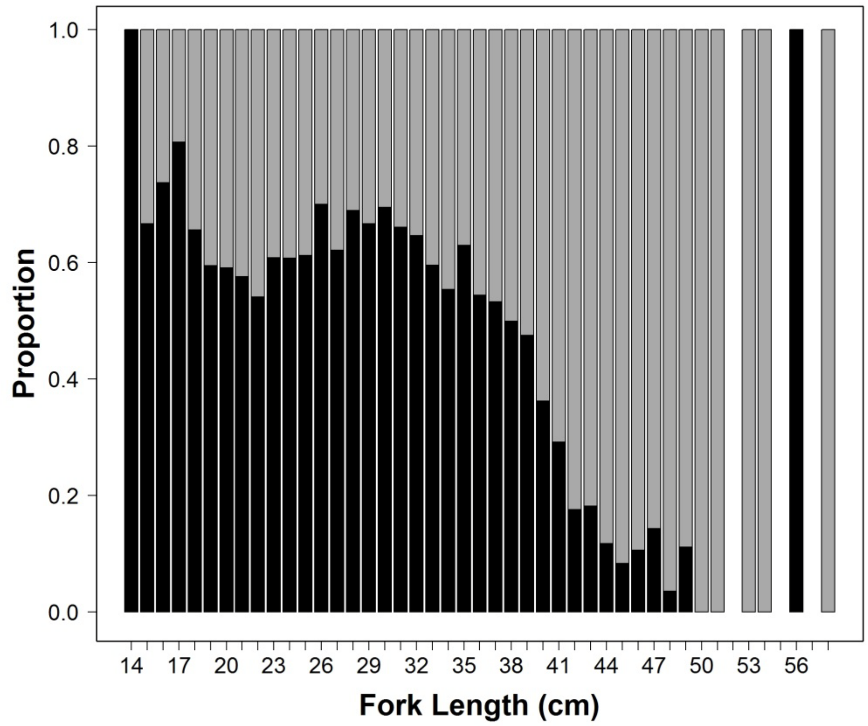

Figure 7. Sex ratio based on fork length. Black bars represent females (total n=4283), gray bars represent males (total n= 3149).

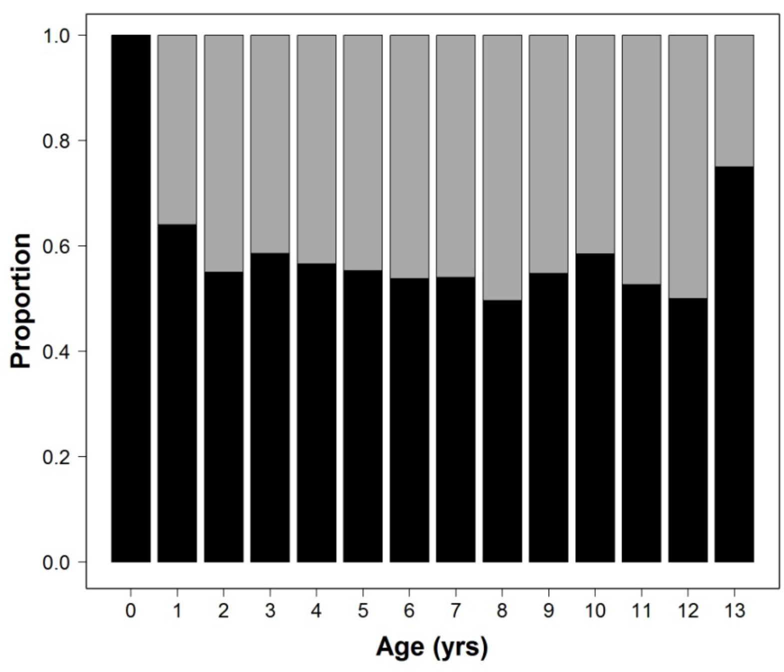

Figure 8. Gray triggerfish age based sex ratio. Female total n= 3892, Male total n= 3118.