A Preliminary Assessment of Reproductive Parameters for Blueline Tilefish in Atlantic Waters from Virginia to Florida

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SEDAR50-DW03

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Executive Summary

Blueline Tilefish analyzed for life history were collected from Virginia to Florida (approximately 24.4^{0}N and 37.5^{0}N), by fishery-independent and fishery-dependent sources throughout 1979 – 2015 (n= 2386 to date). Otoliths of 1019 individuals were assessed for age, which was found to range from 2 to 43 years. If necessary, total length (mm) was converted to fork length (mm) using a meristic conversion from SEDAR 32 (Table 13 in SEDAR 32 - South Atlantic Blueline Tilefish Stock Assessment Report Final Report), producing a range from 307 – 890 mm. The maturity of 2366 reproductive samples was assessed using criteria listed in Brown-Peterson et al. (2011), revealing a spawning season of February – November. Spawning females, with available location data (n=882), were largely collected from South Carolina, Virginia, and North Carolina; however, spawning individuals were found in all states throughout this study. Sex ratio was calculated to be 1.18:1 (F:M), slightly favoring females. Females reach sexual maturity as early as Age 2, as the percentage mature was 100% at Age 2 (n=1), 67% at Age 3 (n=3), 100% at Age 4 (n=13) and Age 5 (n=35), 95% at Age 6 (n=41), and 100% at older ages. Preliminary estimates of age and length at 50% maturity were calculated for the data currently available and ranged from 0.6-1.7 yr and 298-312 mm FL, respectively.

Introduction

Blueline Tilefish (*Caulolatilus microps*) is a commercially and recreationally important fish that is a long-lived, slow-growing, deepwater, demersal species that has historically been described as occurring along the outer continental shelf of North America from Cape Lookout, North Carolina, to Campeche Bank, Mexico (Dooley, 1978). As adults, they appear to settle on the outer continental shelf, shelf break and upper slope, on irregular bottom. At depths of 46 – 256 m, their habitat is known to include ledges, crevices, boulders and rubble piles, where the temperatures range from 9- 23^{0}C (Struhsaker 1969; Ross 1978; Ross and Huntsman 1982; Parker and Mays 1998, Sedberry et al. 2006). Blueline Tilefish have been observed hovering near or entering burrows under rocks (Parker and Ross 1986), a characteristic associated with many Malacanthids (Able et al. 1982; Able et al. 1987; Baird and Baird 1992). Harris et al. (2004) reported a spawning season from February – October, with spawning at night.

Blueline tilefish is an iteroparous gonochorist species that releases eggs in batches for a prolonged period, February through October (Harris et al. 2004); the spawning season may extend beyond October, as no specimens were collected in November and December. Pelagic eggs and larvae of tilefishes (*Caulolatilus* sp. and *Lopholatilus chamaeleonticeps*) have been said to be collected off of the Carolinas and in the northwest Atlantic (Freeman and Turner 1977), while juveniles are said to settle into a more structured habitat within the rocks (Carmichael et al. 2016), where they grow to feed primarily on benthic invertebrates and fishes (Dooley 1978).

In preparation for the benchmark stock assessment planned for Blueline Tilefish in fall 2016, samples were collected from New Jersey to Florida (largely from South Carolina and Virginia), including fishery-independent and fishery-dependent data, in order to assess reproductive parameters in the Blueline Tilefish population(s) along the Atlantic coast of the U.S. Sampling gear consisted of hook and line efforts, chevron traps, short bottom long lines and long bottom long lines. In 2015, a large fishery-dependent sampling effort was made by NMFS using long lines, contributing 827 samples to the dataset. Preliminary datasets from the Southeast Reef Fish Survey (SERFS), and Old Dominion University (ODU) were combined and manipulated respectively into one dataset to evaluate age, sex, maturity, length, and spawning fractions, totaling 2386 individuals for analyses. Combined data from SERFS and ODU dates back to 1979, with ages of 1019 individuals and reproductive histology for 1,728 specimens.

Methods

SERFS (n=1456) fishery-independent sampling (53%) and fishery-dependent sampling (47%)

This study contains samples of Blueline Tilefish that were collected along the eastern coast, from New Jersey to Florida, between 1979 and 2015 (n=2386). These samples were largely collected in South Carolina by the Southeast Reef Fish Survey (SERFS). Note that the collaborative fishery independent snapper grouper monitoring conducted by the Marine Resources Monitoring, Assessment, and Prediction Program (MARMAP), the South East Area Monitoring and Assessment Program-South Atlantic (SEAMAP-SA) (both housed at SC-DNR's Marine Resources Research Institute), and the South East Fishery Independent Survey (SEFIS) (NMFS project housed at SEFSC, Beaufort, NC) are now collectively referred to as SERFS.

Collection of fishery-independent samples (n=773), according to MARMAP protocols (see details in MARMAP 2009; Smart et al. 2015), begins with sorting of species and acquiring weights. Whole fish are sorted and weighed to the nearest gram (g), a pinched, maximum total length (TL) is measured in millimeters (mm), in addition to fork length (FL), and standard length (SL). Otoliths are then removed and stored dry prior to processing, while reproductive tissue is fixed in an 11% seawater-buffered formalin solution to prepare for processing.

Harris et al. (2004) states gear types primarily included snapper/bandit reels, short bottom long lines (SBLL; 20-hook), long bottom long lines (LBLL; 100-hook), and hook and line (H&L), depending on known bottom type (i.e. Hard, rocky, mud, sand). At the fish house, whole weights (g) were taken, TL and sometimes FL (mm), as well as otoliths and reproductive tissue for later processing. For more information concerning these sampling methods, refer to Harris et al. (2004).

Age

Transverse sections of the left sagittal otolith were prepared for aging, when possible, following MARMAP protocol, by first embedding the whole structure in an epoxy resin. One or two transverse sections (0.7–1.0 mm thick) were made through the core with a Buehler Isomet low-speed saw. Sections were mounted on glass slides with Accumount mounting medium and viewed under a dissecting microscope at 20–70X magnification using transmitted light. Increments (one translucent and one opaque zone) were counted independently by two readers who lacked knowledge of specimen length or date of capture. When assignments differed, the readers re-examined the section simultaneously to determine a count.

Reproduction

Following specimen capture and dissection, the posterior portion of the gonads was fixed for 14 days in an 11% seawater–formalin solution and later transferred to 50% isopropanol for an additional 7–14 days. 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 phase were determined without knowledge of capture date, specimen length, or specimen age. Descriptive criteria from Brown-Peterson et al. (2011) were used for determining reproductive phase: immature, developing, spawning, regressing, and regenerating. Independently, two readers determined sex and reproductive phase using histological criteria. When assignments differed, the readers re-examined the section simultaneously to determine reproductive phase. 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, and/or hydration) or postovulatory complexes (POCs).

<u>NMFS (n = 827) 2015 fishery-dependent sampling (100%)</u>

In a collaborative effort, sampling was conducted using generally standardized protocols by cooperating fishermen on industry vessels, with data and biological samples being collected by a trained NMFS-Southeast Fisheries Science Center (SEFSC) fishery observer (SEDAR50-DW02). Sampling largely took place offshore of Virginia, South Carolina, and North Carolina, using SBLL, LBLL, and vertical H&L. Site selection was done during daylight hours, over a general distribution of area, to avoid "clustering" of sampling. Sampling protocol involved species identification, measurement of FL (cm, later converted to mm), otolith removal, reproductive tissue removal, and tissue (pectoral fin clip) collection for genetic analysis.

Otoliths were sent to J. Potts (SEFSC-Beaufort) for processing and analysis.

Reproductive samples were sent to MARMAP/SCDNR for processing and analysis.

Genetic tissue samples were sent to J. McDowell, Virginia Institute of Marine Sciences, for analysis.

For Further detail on the summary of the 2015 Blueline Tilefish cooperative-with-industry data collection project, please refer to SEDAR50-DW02.

<u>ODU sampling (n=272)</u>

Blueline tilefish were collected from the Norfolk Canyon during 2009-2014. Specimens were collected from the commercial and recreational fisheries, as well as from special charters conducted by CQFE and Virginia Marine Resources Commission (VMRC) scientists aboard recreational charter vessels. Recreational samples were primarily collected through the Virginia Marine Sportfish Collection Project, a freezer program conducted by VMRC through which anglers donated carcasses to scientific research after filleting them at local cleaning stations. Length measurements, sagittal otoliths, and macroscopic determinations of sex and reproductive phase were taken for all fish collected. Total weight was measured for all whole fish, and gonads were extracted from fresh specimens, weighed, and placed in 10% formalin for fixation.

Transverse sections of sagittal otoliths were aged using similar methods to those previously described. Aging was attempted for all specimens collected from 2009-2011, as well as a proportionally allocated subsample of the 2012 specimens, based on the 2009-2011 data (Quinn and Deriso, 1999).

Analyses

To date, all SERFS and ODU histological samples have been examined, whereas 80% of 827 samples from NMFS have been examined.

All analyses were done using R Statistics software.

Length: Fork length (mm) was used in analyses when available, or was generated from TL using the meristic conversion from SEDAR 32 (Table 13 in SEDAR 32 - South Atlantic Blueline Tilefish Stock Assessment Report Final Report). This included 352 female specimens.

Reproduction: Immature, Developing, Spawning, Regressing, Regenerating phases were used based on Brown Peterson et al. 2011.

A "State" category was derived from available latitudinal data: Virginia (VA) north of 36.3^0N; North Carolina (NC) 36.3≥Lat>33.5; South Carolina (SC) 33.5≥Lat>32.0; Georgia (GA) 32.0≥Lat>30.4; and Florida (FL) 30.4≥Lat. Latitude values for ODU samples were generated from the NMFS Statistical Area of Capture midpoints for respective sample locations.

There were 2,386 total samples collected between 1979 and 2015. Sex ratio data were analyzed using a Chi-square goodness of fit test to determine if observed ratios differed among geographic areas (states) from an expected 1:1 female:male (F:M) ratio (Zar 1984). Logistic models were used to estimate fork length (L_{50}) and age (A_{50}) at which 50% of the population has reached sexual maturity.

Results

Sex ratio
Table 1 presents a summary of sex ratio by sampling area. Sampling areas were defined by state latitudinal boundaries The total sample size (n=2366) was comprised of 1281 females and 1085 males collected from Virginia through Florida, with most samples collected off South Carolina. The overall female:male sex ratio favored females in all sampling areas except Georgia, which had the smallest sample size (n=15). Only off South Carolina was this female skewed sex ratio significantly different from 1:1. Given the large sample size (n=1337), this statistical significance has no biological significance.

Spawning season and location
From 1979 – 2015, spawning females (n=962) were observed February – November, as seen in Table 3. Immature fish (n=4) were caught individually in the months of March, April, June and September.

Spawning females, with available location data (n=882), were largely collected from South Carolina (568 of 715 specimens), followed by Virginia (170 of 206), and North Carolina (129 of 177); However, spawning individuals were found in all states throughout this study (Table 2). Table 4 provides a monthly count of reproductive phases for each state. Table 5 provides a count of reproductive phases by 5-yr intervals.

Maturity
Female samples (n=1281) were histologically examined to estimate age and size at maturity. There were four immature females in the entire dataset. Females reach sexual maturity as early as Age 2, as the percentage mature was 100% at Age 2 (n=1), 67% at Age 3 (n=3), 100% at Age 4 (n=13) and Age 5 (n=35),

95% at Age 6 (n=41), and 100% at older ages. Age at 50% maturity was calculated using all available data to date. Since the current dataset does not yet include all specimens to be analyzed, we are showing model results and the predicted 50% maturity as a general guideline (Tables 6 and 7).

Spawning fraction

Spawning fraction measures the proportion of mature females spawning daily. For this preliminary analysis of reproductive data, we have not estimated the duration of the spawning indicators and proportionally reduced the fractions in Tables 8-10 to a 24-hr period. These unadjusted results could still be used to examine trends with size (mm FL) and age. The results of both age- and size-based analyses revealed a high spawning fraction overall. With respect to age, the fraction increased from 0.50 at Ages $\leq$ 5 yr to 0.77 at Ages 6-10, and then leveled out at around 0.9 at older ages (Table 9, Figure 1). In addition, there was no evidence for latitudinal variation in spawning fraction. The size-based results did not reveal an increasing trend, but rather a sustained high spawning fraction usually in the range of 0.81-0.89 (Table 10, Figure 2).

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Tables and Figures

Table 1. Sex ratio for Blueline Tilefish, by sampling area, with corresponding p-value denoting level of significance. Sampling areas were defined by state latitudinal boundaries. Data for specimens captured off Georgia were not analyzed due to small sample size.

		State					
Overall		**VA**	**NC**	**SC**	**GA**	**FL**
Females	1281	207	198	739	7	39
Males	1085	190	174	598	8	25
F/M	**1.18**	**1.09**	**1.14**	**1.24**	**0.875**	**1.56**
% Female	**54%**	**52%**	**53%**	**55%**	**47%**	**61%**
Chi Sq	**16.24**	**0.728**	**1.55**	**14.87**		**3.063**
P-Value	**<0.001**	**0.394**	**0.213**	**<0.001**		**0.08**

Table 2. Frequency of reproductive phases of Blueline Tilefish by state. Note: 2 of the 4 immature females had associated catch location data available.

	State					
Repro. Phase	**FL**	**GA**	**NC**	**SC**	**VA**	**Total**
Developing	10	2	15	101	15	143
Immature				1	1	2
Regenerating	10		13	15	5	43
Regressing	2	2	20	30	15	69
Spawning	12	3	129	568	170	882
Total	**34**	**7**	**177**	**715**	**206**	**1139**

Table 3. Frequency of reproductive phases for female Blueline Tilefish by months

						Month							
Maturity	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Total**
Developing	10		4	25	16	10	6	18	10	47	4		150
Immature			1	1		1			1				4
Regenerating	8		1					3	1	29		2	44
Regressing	3			1	1	11	2	15	4	17	10	6	70
Spawning		2	10	53	142	95	97	159	143	213	48		962
Total	**21**	**2**	**16**	**80**	**159**	**117**	**105**	**195**	**159**	**306**	**62**	**8**	**1230**

Table 4. Maturity by state and month for female Blueline Tilefish.

Maturity by State	**Jan**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**
FL							**1**		**33**			**34**
Developing									10			**10**
Regenerating									10			**10**
Regressing									2			**2**
Spawning							1		11			**12**
GA					**2**	**1**	**1**	**3**				**7**
Developing						1		1				**2**
Regressing							1	1				**2**
Spawning					2			1				**3**
NC					**2**	**2**	**8**		**115**	**50**		**177**
Developing							1		10	4		**15**
Regenerating							1		12			**13**
Regressing					1	1	3		11	4		**20**
Spawning					1	1	3		82	42		**129**
SC	**10**	**14**	**53**	**155**	**99**	**22**	**159**	**146**	**57**			**715**
Developing	5	4	20	14	6	3	17	8	24			**101**
Immature			1									**1**
Regenerating	5						2	1	7			**15**
Regressing			1	1	10		11	3	4			**30**
Spawning		10	31	140	83	19	129	134	22			**568**
VA	**11**		**4**	**4**	**12**	**45**	**26**	**5**	**79**	**12**	**8**	**206**
Developing	5		4	2	4							**15**
Immature					1							**1**
Regenerating	3										2	**5**
Regressing	3									6	6	**15**
Spawning				2	7	45	26	5	79	6		**170**
Total	**21**	**14**	**57**	**159**	**115**	**70**	**195**	**154**	**284**	**62**	**8**	**1139**

Table 5. Frequency of reproductive phases for female Blueline Tilefish by year groups.

	Year								
Maturity	**1979 - 1984**	**1985 - 1989**	**1990 - 1994**	**1995 - 1999**	**2000 - 2004**	**2005 - 2009**	**2010 - 2014**	**2015**	**Total**
Developing	35	4	0	41	0	6	16	48	**150**
Immature	2	0	0	1	0	1	0	0	**4**
Regenerating	1	0	0	7	0	0	6	30	**44**
Regressing	13	0	0	12	0	1	21	23	**70**
Spawning	253	114	0	209	12	35	103	236	**962**
Total	**304**	**118**	0	**270**	**12**	**43**	**146**	**337**	**1230**

Table 6. Logistics regression analysis of maturity at age for Blueline Tilefish. Note: 4 immature females with increment counts of: 3, 4, 6, and 6.

Model	**n**	**AICc**	**A_{50}**
Logit Logistic	563	38.44	1.71
Probit Logistic	563	38.51	0.79
cloglog Logistic	563	38.58	0.63

Table 7. Logistic regression analysis of fork length (FL; in mm) at maturity. Note: 4 immature females with FL (mm) of: 307, 312, 320, and 365.

Model	**n**	**AICc**	**L_{50}**
Logit Logistic	1218	28.436	305
Probit Logistic	1218	28.976	298
cloglog Logistic	1218	29.506	301
Cauchy Logistic	1218	31.005	312

Table 8. Female Blueline Tilefish spawning fraction by age.

Age	# Spawners	1st Date Spawn (Month/Day)	Last Date Spawn (Month/Day)	Spawning Season (Days)	# Mature	# Total Spec.	Obs. Maturity	Observed Spawning Fraction (raw data)
2	0				0	1	0.00	
3	1	7/30	7/30	0	2	3	0.67	0.50
4	8	6/13	9/18	97	11	12	0.92	0.73
5	15	3/26	11/26	245	35	35	1.00	0.43
6	24	3/26	9/17	175	38	40	0.95	0.63
7	39	3/26	11/20	239	46	46	1.00	0.85
8	21	4/21	10/30	192	33	33	1.00	0.64
9	28	3/26	10/30	218	36	36	1.00	0.78
10	30	3/26	11/26	245	32	32	1.00	0.94
11	28	2/3	11/26	297	28	28	1.00	1.00
12	35	4/22	10/30	191	39	39	1.00	0.90
13	26	4/7	9/18	164	28	28	1.00	0.93
14	21	4/22	10/30	191	23	23	1.00	0.91
15	15	5/29	11/26	181	19	19	1.00	0.79
16	19	4/30	10/30	183	19	19	1.00	1.00
17	15	4/21	9/18	150	16	16	1.00	0.94
18	13	4/30	9/17	140	14	14	1.00	0.93
19	12	4/7	9/18	164	12	12	1.00	1.00
20	10	4/21	9/17	149	11	11	1.00	0.91
21	12	4/21	9/10	142	13	13	1.00	0.92
22	17	5/29	10/30	154	19	19	1.00	0.89
23	13	3/26	9/18	176	13	13	1.00	1.00
24	5	4/21	9/17	149	8	8	1.00	0.63
25	9	5/29	9/17	111	9	9	1.00	1.00
26	7	4/30	8/23	115	7	7	1.00	1.00
27	5	5/29	9/17	111	6	6	1.00	0.83
28	2	4/21	8/5	106	2	2	1.00	1.00
29	5	5/29	6/29	31	7	7	1.00	0.71
30	6	5/29	9/13	107	6	6	1.00	1.00
31	6	8/4	9/17	44	6	6	1.00	1.00
32	2	6/4	7/16	42	2	2	1.00	1.00
33	2	6/4	9/11	99	2	2	1.00	1.00
34	6	5/22	11/26	188	6	6	1.00	1.00
35	2	4/21	9/11	143	2	2	1.00	1.00
36	2	4/21	8/5	106	2	2	1.00	1.00
37	4	5/29	9/11	105	4	4	1.00	1.00
38	0				0	0		
39	0				0	0		
40	2	5/30	5/30	0	2	2	1.00	1.00
41	0				0	0		
42	0				0	0		
43	1	8/1	8/1	0	1	1	1.00	1.00

Table 9. Female Blueline Tilefish observed spawning fraction by age, using 5 year bins. North and South are split at the VA/NC border.

	Age	# Spawners	# Total Spec	Observed Spawning Frac (Raw data)
Overall	1-5	24	48	0.50
	6-10	142	185	0.77
	11-15	125	137	0.91
	16-20	69	72	0.96
	21-25	56	62	0.90
	26-30	25	28	0.89
	30+	33	33	1
NORTH	1-5	13	18	0.72
	6-10	53	65	0.82
	11-15	22	28	0.79
SOUTH	1-5	9	27	0.33
	6-10	70	97	0.72
	11-15	82	88	0.93
	16-20	60	63	0.95
	21-25	53	59	0.90

Table 10. Female Blueline Tilefish spawning fraction by fork length (FL), with bins center rounding to the nearest 100 mm.

FL mm	# Spawners	1st Date Spawn (Month/Day)	Last Date Spawn (Month/Day)	Spawning Season (days)	# Mature	Spawning Fraction
300	16	4/22	11/26	218	18	0.89
400	136	3/26	11/20	239	219	0.62
500	471	2/5	11/30	299	584	0.81
600	313	2/3	11/30	301	368	0.85
700	21	5/22	11/26	188	24	0.88
800	1	9/3	9/3	0	1	1.00
Total	**962**	**2/3**	**11/30**	**301**	**1218**	**0.79**

Figure 1a. Spawning fraction within increment grouping for female Blueline Tilefish. Number labels above points represent the number of individuals in those specific bins.

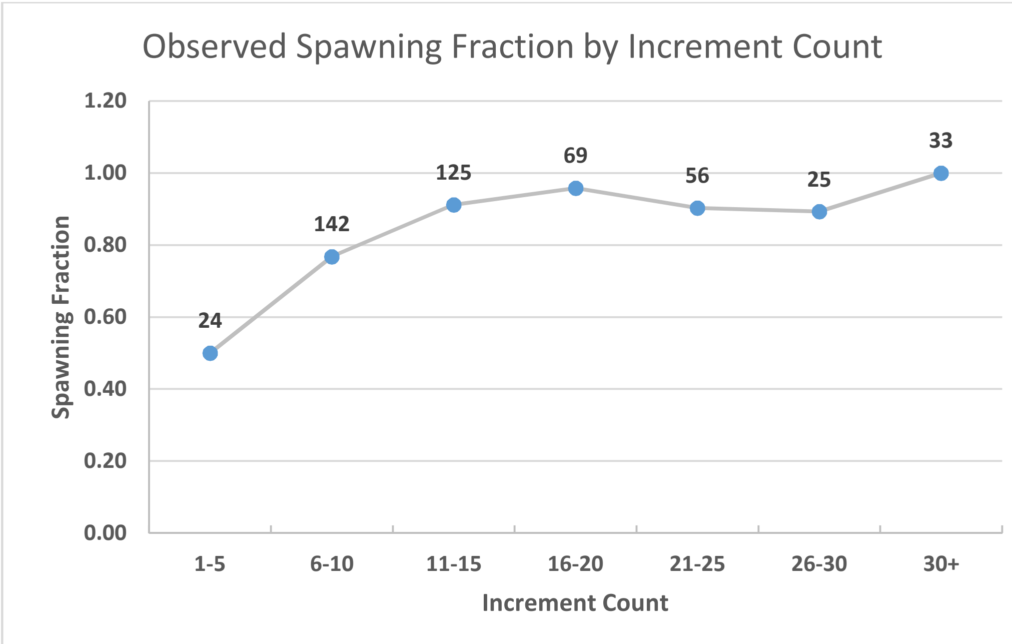

Figure 1b. Spawning fraction within increment grouping for female Blueline Tilefish, including North and South parameters, as delineated from Table 9.

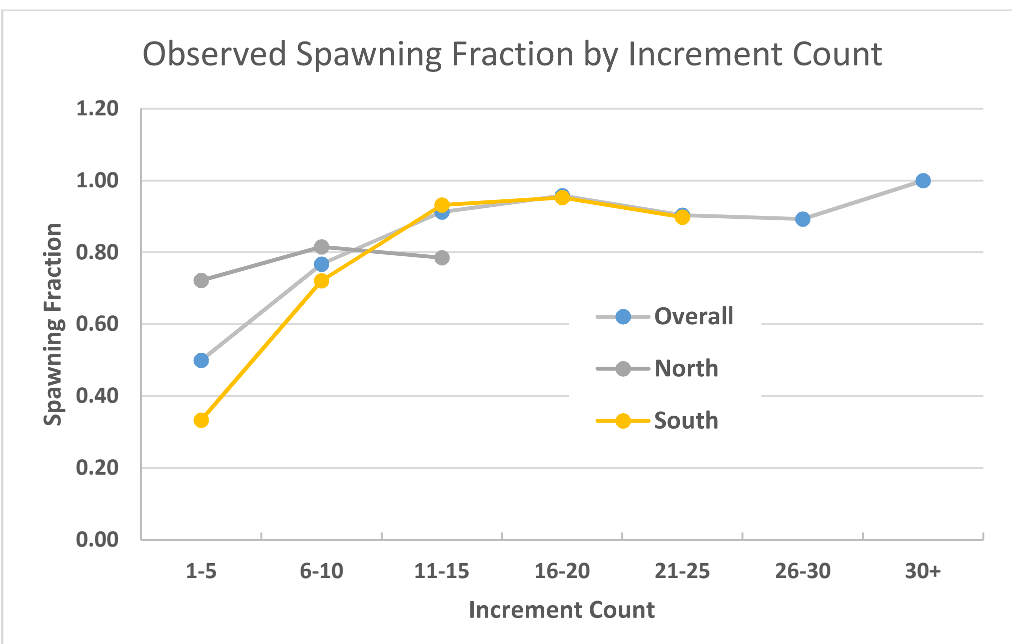

Figure 2. Spawning fraction by binned FL (mm) of female Blueline Tilefish. Number labels above points represent the number of individuals in those specific bins.