Characterization of Reproductive Parameters for Tilefish (*Lopholatilus chamaeleonticeps*) in Atlantic Waters from North Carolina to Florida

Walter J. Bubley, Kevin J. Kolmos, and Matthew Vincent

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Characterization of Reproductive Parameters for Tilefish (*Lopholatilus chamaeleonticeps*) in Atlantic Waters from North Carolina to Florida

SEDAR89 – WP – 03
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Prepared by Walter J. Bubley[1], Kevin J. Kolmos[1], and Matthew Vincent[2]

[1] Marine Resources Research Institute; South Carolina Department of Natural Resources; P. O. Box 12559; Charleston, SC 29422
[2] NOAA Fisheries, Southeast Fisheries Science Center, NOAA Beaufort Lab; 101 Pivers Island Rd; Beaufort, NC 28516

MARMAP/SEAMAP-SA Reef Fish Survey Technical Report #2024-05

Executive Summary

Tilefish analyzed for life history were collected from Virginia to Florida (approximately between 37.2°N and 24.3°N), by fishery-independent and fishery-dependent sources throughout 1977 – 2022 (n= 7,069). Maximum total length (mm) (range 310 – 1155 mm) was utilized throughout analyses and was later converted to age using the mean size at age from the von Bertalanffy curved developed for SEDAR89 (L = 731.53; k = 0.2507; t_0 = -0.5 (fixed)). The reproductive phase of 6,959 samples from males and females was assessed using criteria listed in Brown-Peterson et al. (2011). Based on multiple criteria, there was no evidence indicating that Tilefish in the western north Atlantic Ocean were hermaphroditic. Females reached sexual maturity as small as 329 mm TL and young as 2 years old, with the largest/oldest immature individuals being 575 mm TL and 11 years old, respectively. Females were collected in all months, with spawning indicators present from January – November, excluding October and the entire spatial range. However, spawning fraction varied by month and length of fish, which needed to be addressed when calculating total fecundity. First, a modeled female length at maturity curve was created using a binomial model with the logit link. Then, spawning frequency was estimated with a model of spawning indicators that integrated differences in spawning fraction and spawning season duration by maximum total length, which was then converted to age. Finally, total fecundity at age was the product of batch fecundity and spawning frequency for mean length at age. The spawning frequency during the year ranged from 0 – 48 in mature female Tilefish from 1 to 20+ years old, with a total annual fecundity ranging from 0 – 3.5 million eggs.

Introduction

Tilefish (*Lopholatilus chamaeleonticeps*) is a commercially and recreationally important fish that is a long- lived, slow-growing, deepwater, demersal species that historically has been described as occurring along the outer continental shelf from Nova Scotia to Suriname, including the Gulf of Mexico and continental Caribbean Sea (Bigelow and Schroeder 1953; Dooley 1978) Tilefish is an iteroparous species that releases eggs in batches for a prolonged period (Grimes et al. 1988), but there has been a question of whether the population in the Atlantic waters off the southeastern U.S. was gonochoristic or hermaphroditic based on findings from the Gulf of Mexico (Lombardi et al. 2010; Lyon 2010). Female maturity has been characterized in previous assessments (SEDAR 4; SEDAR 25; SEDAR 66), but the recent formation of the cooperative South Atlantic Deepwater Longline (SADL) survey has provided a large amount of life history samples from an extensive spatial range, warranting a re-examination of the parameters input into the model. Additionally, gonad weight was used as a proxy of reproductive potential in previous assessments due to limited data. However, total fecundity is a more direct representation if the data are available (SEDAR 66). Variables necessary to calculate total annual fecundity need to be explored, such as batch fecundity, spawning fraction (i.e. the proportion of females with spawning indicators), and spawning season duration, as they may be affected by fish size or time of year (Fitzhugh et al. 2012; Klibansky 2015).

Objectives

1. Characterize reproductive strategy of Tilefish in the Atlantic waters of the southeastern U.S.
2. Develop maturity ogives to describe female Tilefish maturity in the Atlantic waters of the southeastern U.S.
3. Estimate batch fecundity of female Tilefish in the Atlantic waters of the southeastern U.S., taking into account size/age differences.
4. Estimate total annual fecundity of female Tilefish in the Atlantic waters of the southeastern U.S., taking into account size/age and temporal differences in spawning frequency.

Methods

Sampling

Tilefish were collected as a part of several studies overseen by the Reef Fish Survey group within the South Carolina Department of Natural Resources. Fishery-independent and fishery-dependent sampling collection occurred from 1977- 2022. Collection gears included hook and line, snapper reel, experimental trap, kali pole, and a variety of longlines (short bottom, long bottom, commercial, and cooperative projects CRP and SADL). Fishery-independent collections were taken following MARMAP/SERFS protocols (Smart et al. 2015). Attempts were made to collect fish from all months and latitudes within the South Atlantic Fishery Management Council Jurisdiction. All fishery-independent caught fish were weighed to the nearest gram (g) and measured in millimeters (mm) for a pinched tail maximum total length (TL), in addition to fork length (FL), and standard length (SL). Fishery-dependent samples had whole weights (g) and length measurements taken (TL and sometimes FL (mm))

Life History Processing

In the field, otoliths and gonad samples were taken from female Tilefish following MARMAP/SERFS protocols (Smart et al. 2015). Fecundity samples were taken from individuals with ovaries that had oocytes undergoing maturation (stage-2 and stage-3 yolked oocytes) but prior to ovulation (Hunter et al. 1992). For fecundity, whole ovaries were weighed to the nearest gram and depending on the size of the gonad, stored whole or a portion, in a 10% seawater-buffered formalin solution. Fresh and preserved gonad weights were obtained for some ovaries so a regression equation could be developed to convert fresh weight to preserved weight. When the gonad was not appropriate for fecundity, it was prepared for just histological processing and sex/phase assignment; either the whole or posterior portion, depending on the size, was fixed in an 11% seawater-buffered formalin solution.

At the lab, fecundity processing followed MARMAP/SERFS protocols (Smart et al. 2015). In summary, the preserved whole gonad or portion was weighed, two or three 150-200 mg subsamples of the ovarian tissue were taken, oocytes were manually separated from non-egg tissue, and stage-3 oocytes from these subsamples were counted. Original subsample weights were considered too small for use in batch fecundity estimates as per discussions during SEDAR 4 and SEDAR 25, so larger subsample weights and new egg counts were taken to compute new batch fecundity estimates. Attempts were made to obtain specimens from the full size range of mature female Tilefish.

At the lab, histological processing followed MARMAP/SERFS protocols (Smart et al. 2015). Briefly, tissue was dehydrated, infiltrated with paraffin, embedded in paraffin, and transverse sectioned (6-8 µm thick) prior to mounting on slides and staining with double strength Gill hematoxylin and eosin-y. Sex and reproductive phase was then assigned by two readers independently without knowledge of capture date, specimen length, or specimen age using histological criteria from Brown-Peterson et al. (2011). When assignments differed, the readers re-examined the sections simultaneously to reach consensus. If consensus could not be reached, then no sex and/or phase was assigned.

Life History Analysis

Reproductive strategy was characterized using criteria from Sadovy and Shapiro (1987) to determine if Tilefish were hermaphroditic in the Atlantic waters off the southeastern U.S. The criteria consisted of population demographics, gonadal structure, germ cell remnants, and transitional individuals. The fifth criteria involved experimental induction of sex change, which was not possible with this species. Not one of these criteria individually are determinative of the reproductive strategy, but the preponderance of evidence for or against hermaphroditism from these four categories may allow for an informed decision to be reached. To assess population demographics, age-frequency distribution by sex was examined. Age was chosen instead of length due to the sexual dimorphism exhibited in this species (Grimes et al. 1988). If Tilefish were sequentially hermaphroditic, it would be expected that one sex would dominate the frequency of one tail of the distribution, while the other sex would dominate the frequency of the other tail. To assess gonadal structure, histological sections of male Tilefish were examined for the presence of an ovarian lumen, which would provide evidence of protogyny. To assess germ cell remnants, histological sections were examined for remnants of female tissue in males or vice versa, with a particular focus on vitellogenic or atretic oocytes in males. Finally, to assess transitional individuals, histological sections were examined for degenerating tissue of one sex and proliferating tissue of another.

Maturity analysis was performed for only female Tilefish. Fish were considered mature using the traditional definition which included all months and individuals with oocyte development at or beyond the cortical alveolar stage or specimens with beta, gamma, or delta stages of atresia (Hunter and Macewicz 1985). Tilefish maturity at length data were modeled using a GLM with the following equations:

$$y_i \sim Bernoulli(n_i)$$
$$n_i = f(c + d * TL_i) \qquad (1)$$

y_i is the binary response variable where 1 indicated mature and 0 indicates immature for individual i, n_i is the predicted probability of maturity that is modeled as a linear function of maximum total length (TL_i) with parameters c and d. Three link functions (f) were tested and compared: logit link, probit link, and Cauchy link. The best fit model was selected by AIC and used to predict the maturity at length for tilefish.

For batch fecundity analysis, counts were converted from the preserved subsample weight to preserved whole gonad weight for estimates of batch fecundity. Any individual in which we did not have a preserved whole gonad weight, we applied a conversion developed for this study (Preserved wt (g) = fresh wt (g) * 0.9246 – 3.9757; n = 26; adj. r^2=0.993; fig. 1). Batch fecundity to TL (mm) was explored with regression analysis using a linear approach, power function, and log transformations. The approach with the lowest AIC value was selected.

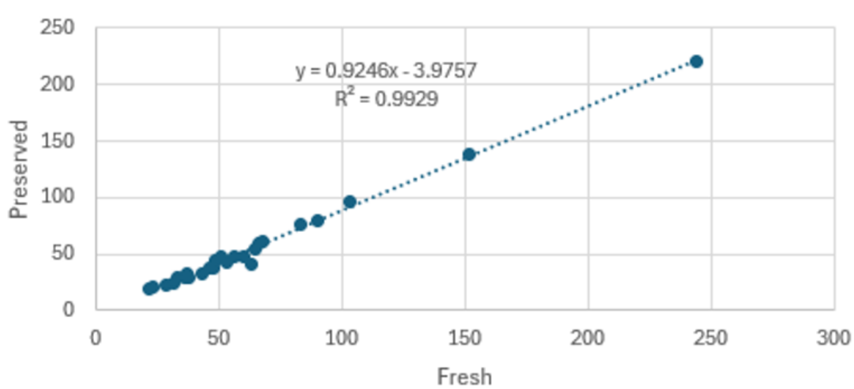

Figure 1. A regression between preserved ovary weight (g) and fresh ovary weight (g) for conversion during batch fecundity calculations if preserved whole ovary weight was not available. The linear equation and r^2 values are included.

To determine spawning frequency (i.e. number of batches spawned during a year) and peak spawning date, mature female Tilefish were selected from the available histological data. 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 (MNO), and/or hydration (HO)) or postovulatory follicle complexes (POCs) and were given a response value (y_i) of 1; those fish that were not spawning were given a response value (y_i) of 0. Presence/absence of spawning indicators by date was fit to a plateau model that can be used to determine the spawning frequency and peak spawning. Binary response data of spawning indicators were modeled by a Bernoulli process where the probability (p_i) of spawning capacity was a function of day of the year (1-365).

$$y_i \sim Bernoulli(p_i)$$
$$p_i = \frac{1}{1+e^{-\frac{DAY-m}{s}}} - \frac{1}{1+e^{-\frac{DAY-(m+d)}{s}}} \tag{2}$$

In the equation above m is the day when spawning capacity occurs in 50% of the population; s is the slope of the plateau that shows variation around m and represents the asynchrony in the start of spawning among females; d is the average duration for which spawning indicators are present (i.e., average spawning season duration). This model can be extended to include linear relationships between fish length and the start of spawning ($m1$), as well as fish length and the duration of spawning ($d1$).

$$p_i = \frac{1}{1+e^{-\frac{DAY-m-m1*TL}{s}}} - \frac{1}{1+e^{-\frac{DAY-(m+m1*TL+d+d1*TL)}{s}}} \tag{3}$$

A positive value in $m1$ indicates an earlier start day in the year with increasing fish length and a positive value in $d1$ indicates longer spawning season duration with fish length. In the above equation, the day when there is 50% spawning indicators by fish length is $m + m1 * TL$ and the average spawning duration by fish length is $d + d1 * TL$. An additional extension of the model links the maximum total length through a logistic relationship to the proportion spawning.

$$p_i = \frac{\frac{1}{1+e^{-r(TL-l)}}}{1+e^{-\frac{DAY-m-m1*TL}{s}}} - \frac{\frac{1}{1+e^{-r(TL-l)}}}{1+e^{-\frac{DAY-(m+m1*TL+d+d1*TL)}{s}}} \tag{4}$$

In this equation r is the slope of the logistic curve by fish length and l is the fish length at which half of the population has spawning indicators. This equation is similar to multiplying the duration of spawning season by a logistic model fit to the spawning capable individuals by fish length, but this model has the added benefit of accounting for the seasonality in spawning and sampling interactions. An alternative formulation of the model assumes that the duration of the spawning season increases linearly with fish total length (w) both in the start date and end date. In this model the peak spawning date is constant across fish lengths, which is not the case for equations (3) and (4).

$$p_i = \frac{1}{1+e^{-\frac{DAY-m+w*TL}{s}}} - \frac{1}{1+e^{-\frac{DAY-(m+w*TL+d)}{s}}} \tag{5}$$

Varying complexities of models were tested by setting different parameters to 0 and comparing AIC and BIC values. The model with the lowest AIC was chosen as the preferred model. The spawning frequency at length can be calculated by integrating across a year (i.e., 1 to 365).

Spawning indicators (HO, POC, and MNO) were estimated to last 60 hours based on the temperature (mean = 10.5ºC) at which spawning female Tilefish were collected and the duration of spawning indicators in Northern Anchovy (Hunter and Macewicz 1985a, b), a species that spawns at a similar temperature range (13 - 19°C). Therefore, a correction factor of 0.4 (i.e., 60/24) was applied to the spawning frequency to convert to a daily rate.

Calculation of peak spawning date depends on the parameterization of the plateau model. For the basic 3 parameter plateau model, the peak spawning can be calculated as $m + \frac{d}{2}$. Models that have linear relationships of starting date and/or duration with fish length will have different dates of peak spawning by fish length calculated by $m + m1 * TL + \frac{d+d1*TL}{2}$. For the model where the start and end date increased linearly with fish length, the peak spawning duration can be calculated in the same manner as the 3-parameter plateau model (i.e., $m + \frac{d}{2}$). Spawning frequency at length is calculated as the area under the plateau model curve given a length (i.e., integrating from 1 to 365 days).

Total Fecundity is calculated as the product of the spawning frequency and the batch fecundity. Von Bertalanffy growth curves fit to female data were used to determine the mean length at age. These lengths were then inserted into the regression equations for the batch fecundity and the plateau model to determine spawning frequency to calculate the total fecundity. Maturity at age was determined by inserting the mean length at age into the logistic regression of maturity at length. The reproductive output at age was determined by multiplying the maturity at age by the total fecundity at age.

Table 1. Growth curve parameters for golden tilefish with standard errors of parameters in parenthesis.

Data Source	L_∞	k	t_0	CV
All Data	830.18 (50.55)	0.2048 (0.043)	-0.5 (fixed)	0.16 (0.03)
Male	950.5 (133.58)	0.1645 (0.049)	-0.5 (fixed)	0.14 (0.05)
Female	731.53 (67.0)	0.2507 (0.072)	-0.5 (fixed)	0.13 (0.05)

Results and Discussion

Sampling

From 1972 to 2022, the SCDNR Reef Fish Survey group collected, processed, and staged reproductive tissue from 7,069 individual fish (female = 2,697 and male = 4,372) from fishery-independent and fishery-dependent sources over all 12 months of the year (fig. 1). The sizes ranged from 310 mm TL to 1,155 mm TL for males and 329 mm TL to 1,000 mm TL for females. The ages ranged from 2 to 34 years of age for males and 2 to 41 years of age for females. These were obtained from 24° to 37° N latitude, which encompasses the entirety of the jurisdictional boundary of the South Atlantic Fishery Management Council and the stock boundary for assessments. Ovarian tissue which could be used for fecundity analyses were collected from 65 individuals between 1996 and 2009 from February – June, which ranged in length from 468 mm TL to 791 mm TL.

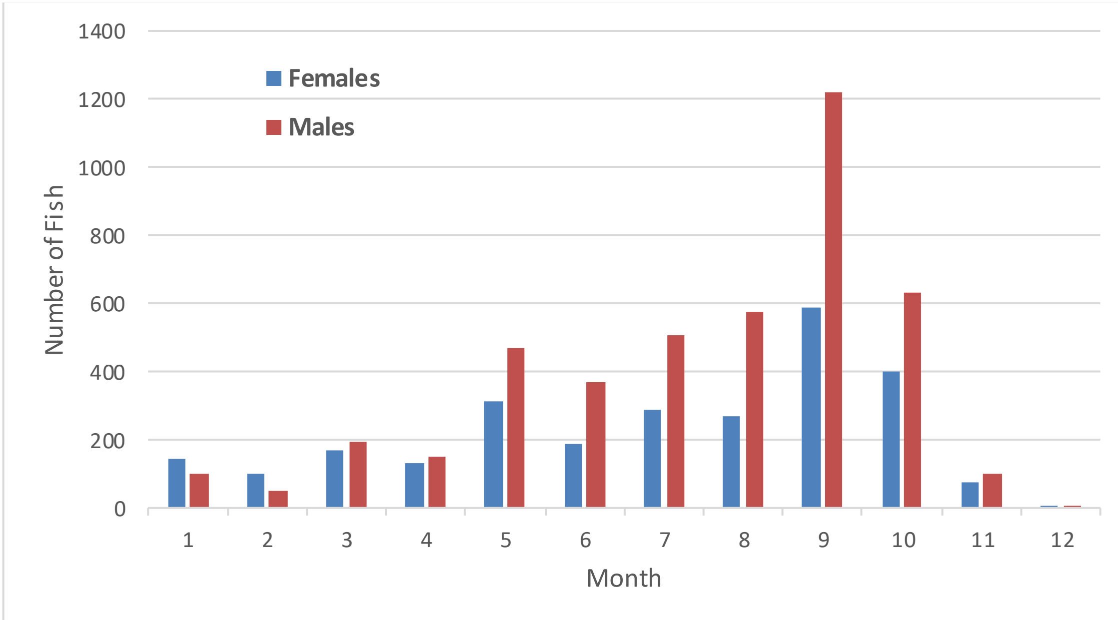

Figure 1. Sample size of collected Tilefish for histological processing by sex and month utilized in this reproductive analysis.

Reproductive Strategy

Of the four criteria examined to characterize hermaphroditism in fish, none of them indicated Tilefish are hermaphroditic. The age frequency distribution did not show substantial disparity between the sexes (fig. 2), most notably in the left tail of the distributions, where you would expect one sex to be more prevalent if sequential hermaphroditism was the reproductive strategy. Regarding gonadal structure, there were no individuals that retained an ovarian lumen out of the 4,372 histological samples from males that were examined. When examining germ cell remnants, there were no females with testicular remnants (n = 2,697). There were however 1% of males (n = 44) which had previtellogenic oocytes, but there were no instances of vitellogenic or atretic oocytes, which would indicate viable eggs and thus functionality. Sadovy and Shapiro (1987) indicated that the presence of previtellogenic eggs in testicular tissue is the weakest indication of hermaphroditism as there are common causes that may produce this occurrence. However, the presence of previtellogenic eggs in testes does not necessarily translate into functionality, which is the definition of hermaphroditism relevant to fish populations (Atz, 1964; Yamamoto, 1969; Takahashi, 1977; Takahashi and Shimizu, 1983). Finally, there was a complete lack of transitional individuals from those examined histologically from both sexes (n = 7,069) even though fish were collected in all months of the year. Taken together, Tilefish do not meet any of the four criteria to be classified as hermaphroditic in the Atlantic waters off the southeastern U.S., which would indicate this is a gonochoristic population.

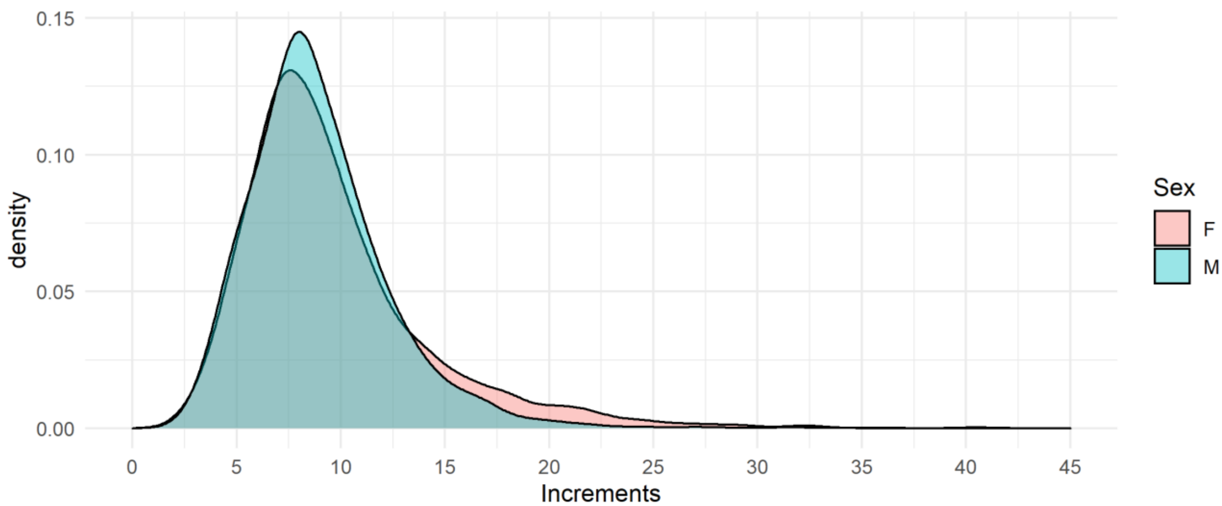

Figure 2. Density plot of age (increment) distribution of Tilefish by sex.

Maturity

Female Tilefish samples (n= 2,673) were histologically examined to estimate size at maturity. There were 47 individuals collected that were classified as immature females. Female Tilefish reached sexual maturity as small as 329 mm TL and young as 2 years old, with the largest/oldest immature individuals being 575 mm TL and 11 years old, respectively. Model fits were reasonable for all link functions, but the model with the lowest AIC was achieved by the logit link (Table 2). The maturity at length logit link model was used to estimate the maturity at age (Table 4). These estimates were similar to a Cauchy model fit to maturity-at-age data (not shown).

Table 2. Parameter estimates, negative log likelihoods, and AIC for binomial regressions of total length on proportion mature for different link functions.

Model	Intercept	Slope	Log Likelihood	Delta AIC
Logit	-7.24	0.022	164.91	0
Probit	-2.87	0.0095	166.01	2.2
Cauchy	-45.6	0.12	178.07	26.3

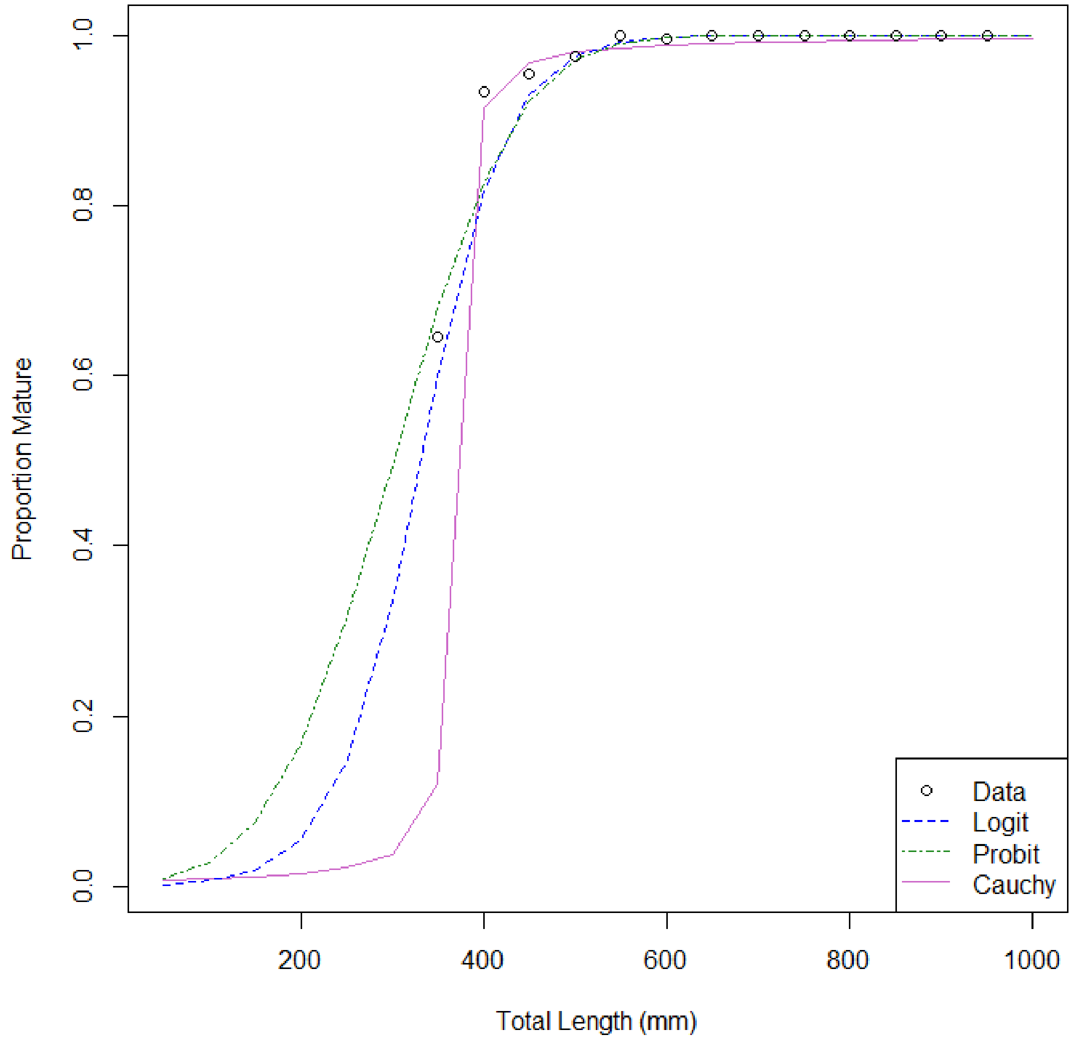

Figure 3. Proportion mature predicted from observed maximum total length in millimeters where the data points are pooled in 50 mm bins and the different lines indicate different link functions for the binomial model.

Batch Fecundity

The log transformation of both batch fecundity and TL (ln(Batch fecundity) = a +b*ln(TL)) produced the best AIC value compared to the power function and linear regression (Table 3; Figure 4). This produced an equation for batch fecundity by size which could be applied to the mean length at age and provides a batch fecundity at age (Table 5).

Table 3. Results from the selected batch fecundity equation.

Dependent Variable	Range	Intercept (a)	SE_a	slope (b)	SE_b	Adj. R2	F	n
TL (mm)	468-791	-12.362	3.656	3.574	0.567	0.38	39.8	65

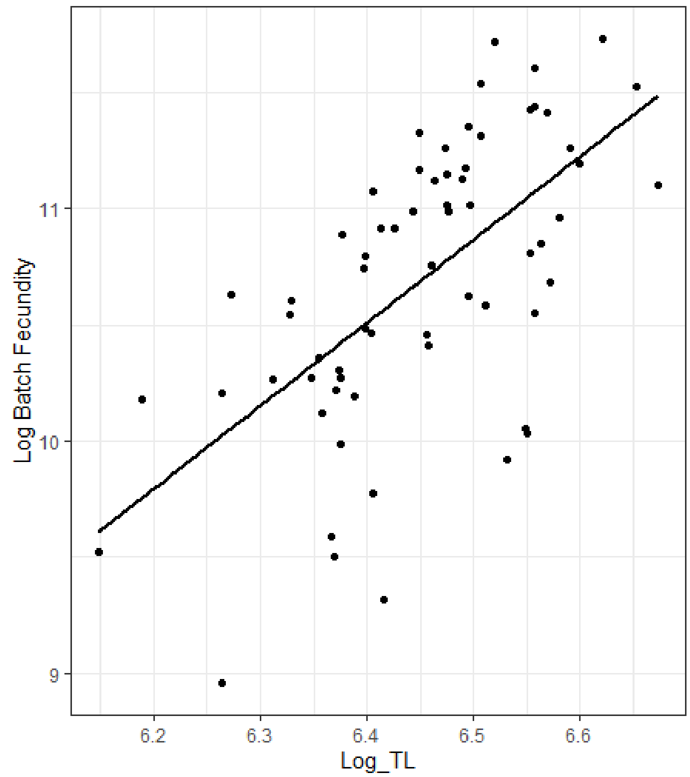

Figure 4. Fit of log transformed batch fecundity to log transformed TL of female Tilefish.

Spawning Frequency and Peak Spawning Date

Numerous models of varying complexity were fit to the spawning indicators by length and day of year through the plateau model. The model selected by AIC had spawning season duration linearly related to total length and spawning fraction logistically related to total length (i.e., the model that estimated the m, s, d, d1, r, and l parameters). This model was also the second lowest BIC value, indicating that this model had similar support using these criteria as the lowest BIC model (Table 4). Plots of fits to a selection of the plateau models are presented in Figures 5-7.

Table 4. Parameters estimates, negative log likelihood and difference in AIC and BIC from the model with the lowest relative value. Parameters with no entry indicate the parameter was not estimated in the model.

m	m1	s	d	d1	w	r	l	Log Likelihood	Delta AIC	Delta BIC
80.37		16.12	102.53					641.80	172.55	156.92
79.72		14.01	9.89	0.148				562.90	16.76	6.96
80.19		14.00		0.16				563.79	16.54	0.91
18.07	0.092	15.01	106.73					604.86	100.68	90.88
124.70	-0.068	13.95	-32.53	0.213				556.81	6.58	2.61
	0.115	14.99	85.24	0.039				602.69	96.35	86.54
99.10	-0.030	13.99		0.164				559.42	9.80	0.00
	0.113	16.85		0.169				661.83	212.63	197.00
98.63	-0.031	13.18	10.00	0.154		0.026	437.13	551.64	0.24	7.93
77.63		12.94	33.69	0.119		0.024	443.10	552.52	0.00	1.86
21.68	0.080	12.41	117.36			0.021	464.65	566.09	27.15	29.00
76.18		13.16	116.11			0.022	475.89	591.60	76.18	72.21
161.76		14.18	-56.56		0.125			561.83	14.61	13.77
132.88		14.49			0.082			572.24	33.44	4.81
147.82		13.73	-28.07		0.105	0.029	432.05	558.47	11.91	17.81

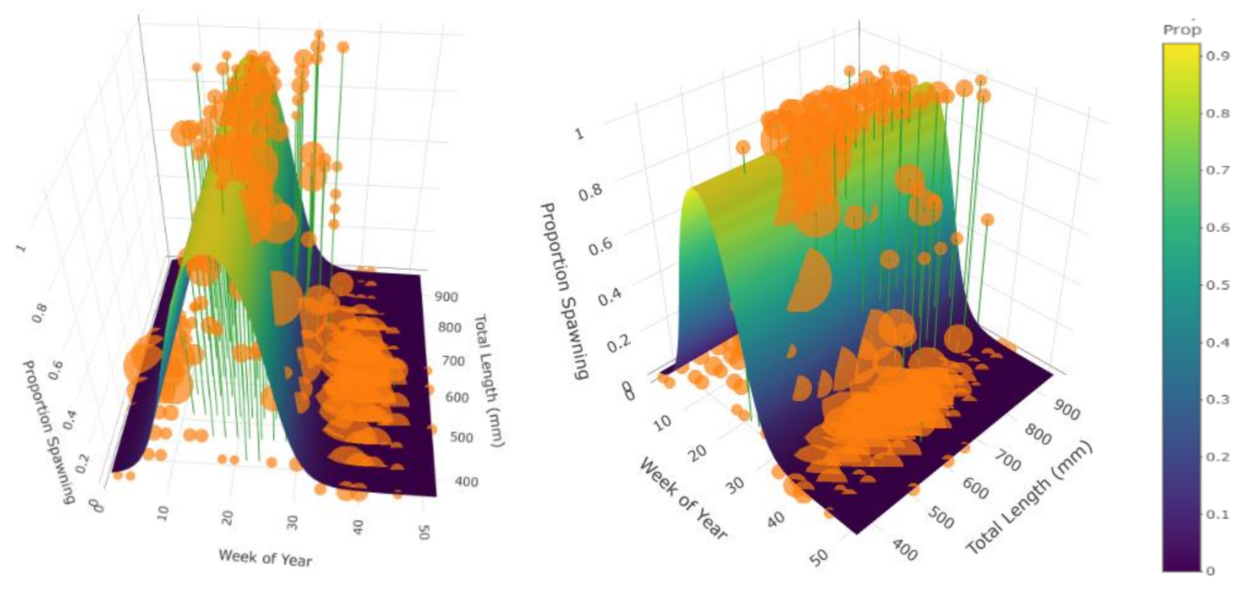

Figure 5. Plot of proportion spawning by total length in millimeters and week of the year viewed from multiple angles. The orange bubbles indicate the proportion of samples spawning binned by 50 mm and 2-week intervals, where the size of the bubble is relative to the number of samples and are connected to the xy-plane by a green line. The surface is the proportion spawning predicted by the 3-parameter plateau model where higher proportion spawning are a lighter color.

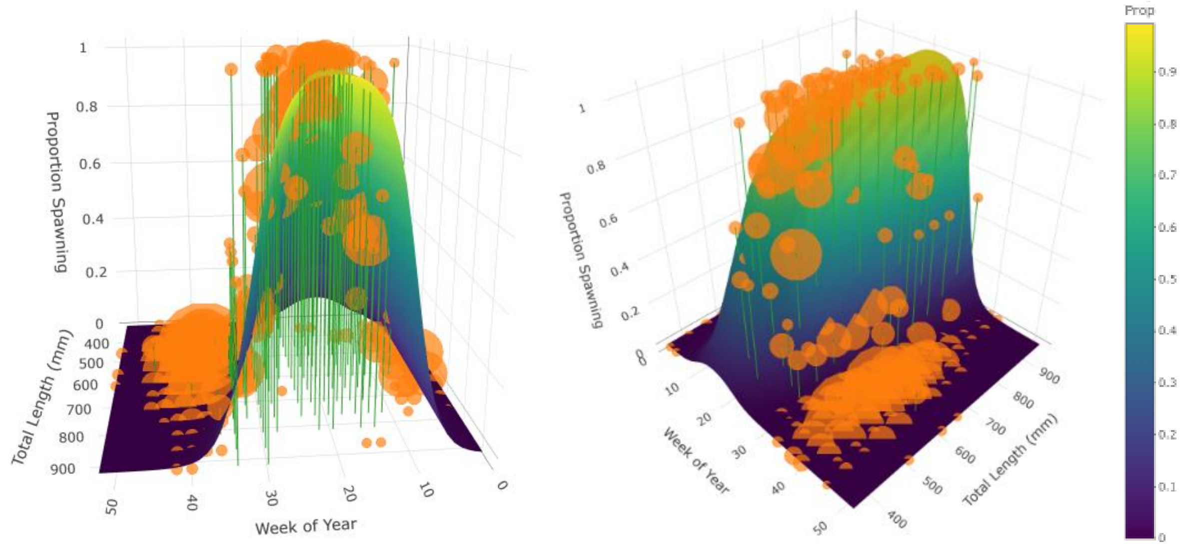

Figure 6. Plot of proportion spawning by total length in millimeters and week of the year viewed from multiple angles. The orange bubbles indicate the proportion of samples spawning binned by 50 mm and 2-week intervals, where the size of the bubble is relative to the number of samples and are connected to the xy-plane by green lines. The surface is the proportion spawning predicted by the model selected by AIC (i.e., estimating the m, s, d, d1, r, and l parameters), where higher proportion spawning are a lighter color.

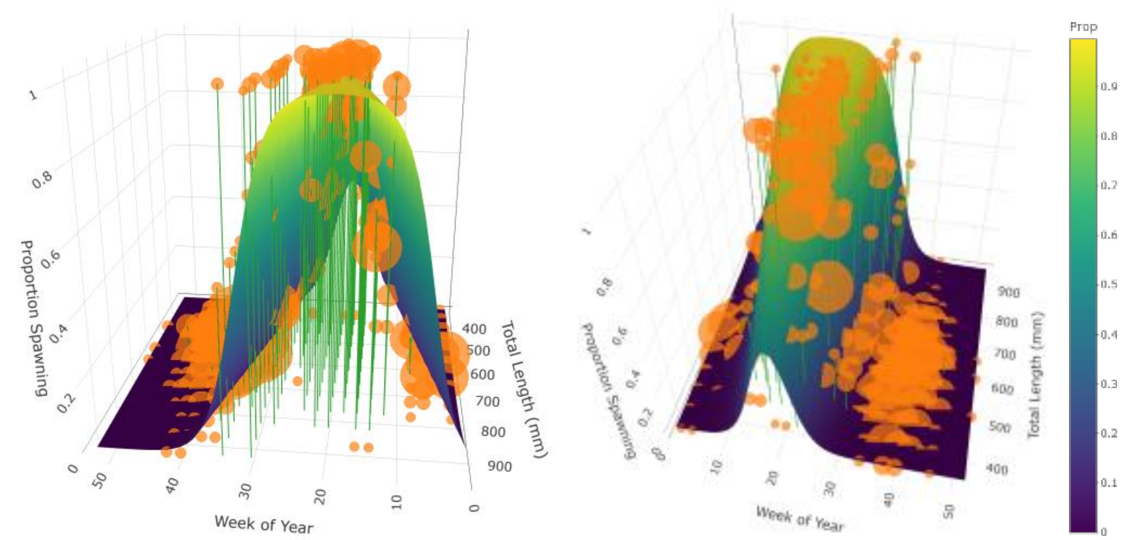

Figure 7. Plot of proportion spawning by total length in millimeters and week of the year viewed from multiple angles. The orange bubbles indicate the proportion of samples spawning binned by 50 mm and 2-week intervals, where the size of the bubble is relative to the number of samples and are connected to the xy-plane by green lines. The surface is the proportion spawning predicted by the model where spawning season duration increased linearly with length in both directions around a common peak date (i.e., estimated parameters m, s, d, and w), where higher proportion spawning are a lighter color.

The date of peak spawning was calculated from the plateau model selected by AIC for each mean length at age from the female growth curve and then averaged across ages. The peak spawning was estimated to be on May 15th (the 135th day or 0.37 fraction of the year).

Total Fecundity

The number of batches predicted by the integration over the year of the plateau model by proportion spawning were rounded to the nearest integer for the model selected by AIC (Table 5). Maturity at age was predicted by the logit link model from the mean total length at age. Batch fecundity at age was calculated from the exponential function of mean length at age. Total Fecundity was the product of batch fecundity and spawning frequency, while the total reproductive output is the product of total fecundity and maturity. This method was compared to calculating total fecundity by binning by size or age and produced similar values (not shown here), but the plateau model operated with continuous fish lengths and spawning season durations, providing higher resolution data.

Table 5. Mean length at age for females, maturity at age, batch fecundity at age, spawning frequency, total fecundity and total reproductive output for Tilefish.

Age	Female Length	Maturity	Batch Fecundity	Spawning Frequency	Total Fecundity	Total Reproductive Output
1	229.31	0.1	1168	0	0	0
2	340.68	0.55	4807	2	9614	5281
3	427.36	0.89	10807	14	151301	134662
4	494.82	0.97	18247	29	529166	514596
5	547.31	0.99	26164	37	968061	959430
6	588.17	1	33841	40	1353636	1348666
7	619.96	1	40847	42	1715557	1712406
8	644.7	1	46979	44	2067058	2064845
9	663.96	1	52189	45	2348514	2346862
10	678.94	1	56523	46	2600049	2598730
11	690.6	1	60070	46	2763225	2762138
12	699.68	1	62940	47	2958161	2957207
13	706.74	1	65240	47	3066280	3065433
14	712.24	1	67072	47	3152375	3151602
15	716.52	1	68523	48	3289090	3288355
16	719.85	1	69667	48	3344030	3343336
17	722.44	1	70568	48	3387245	3386580
18	724.45	1	71274	48	3421152	3420510
19	726.02	1	71827	48	3447710	3447085
20	727.24	1	72260	48	3468480	3467867

Literature Cited

Atz, J. W. 1964. Intersexuality in fishes, p. 145-232. In: Intersexuality in vertebrates including man. C. N. Armstrong and A. J. Marshall (eds.). Academic Press, London, England.

Bigelow, H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildl. Serv., Fish. Bull. 53. 577 p.

Dooley, J.K. 1978. Systematics and biology of the tilefishes (Perciformes: Brachiostegidae and Malacanthidae), with descriptions of two new species. NOAA Tech. Rep. NMFS Circ. 411. 78 p.

Grimes, C.B, Idelberger, C.F., Able, K.W., and Turner, S.C. 1988 The reproductive biology of tilefish, Lopholatilus chamaeleonticeps (Goode and Bean), from the United States Mid-Atlantic Bight, and the effects of fishing on the breeding system. Fishery Bulletin. 86(4): 745-762.

Lombardi, L., Fitzhugh, G. and Lyon, H. 2010. Golden Tilefish (Lopholatilus chamaeleonticeps) age, growth, and reproduction from the northeastern Gulf of Mexico: 1985, 1997-2009. SEDAR22-DW-01. SEDAR, North Charleston, SC. 35 pp.

Lyon, H. 2010. Evidence of hermaphroditism in Golden Tilefish (Lopholatilus chamaeleonticeps) in the Gulf of Mexico. SEDAR22-DW-14. SEDAR, North Charleston, SC. 4 pp.

Hunter, J.R. and Macewicz B.J. 1985a. Measurement of spawning frequency in spawning fishes. In: Lasker R (ed) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fishes: Application to the Northern Anchovy, Engraulis mordax. NOAA Tech Rep NMFS 36:79–94.

Hunter, J.R. and Macewicz B.J. 1985b. Rate of atresia in the ovary of captive and wild northern anchovy, Engraulis mordax. Fishery Bulletin 83:119–136.

Hunter, J.R., Macewicz, B.J., Lo, N.C., and Kimbrell, C.A. 1992. Fecundity, spawning, and maturity of Dover sole, *Microstomus pacificus*, with an evaluation of assumptions and precision. Fishery Bulletin 90:101-128.

Klibansky, N. 2015. Estimates of reproductive activity in red snapper by size, season, and time of day with non-linear models. SEDAR41-DW49. SEDAR, North Charleston, SC. 22 pp.

Sadovy, Y., anf Shapiro, D. Y. 1987. Criteria for the Diagnosis of Hermaphroditism in Fishes. Copeia, 1987(1): 136–156. https://doi.org/10.2307/1446046

SEDAR4, 2004. SEDAR 4: Stock assessment of the deepwater snapper-grouper complex in the South Atlantic. SEDAR, North Charleston, SC.

SEDAR 25, 2011. SEDAR 25: South Atlantic Tilefish. SEDAR, North Charleston, SC.

SEDAR 25 Update, 2016. SEDAR 25: Stock Assessment of Golden Tilefish off the Southeastern United States, 2016 SEDAR Update Assessment. SEDAR, North Charleston, SC.

SEDAR 66, 2021. SEDAR 66: South Atlantic Tilefish Stock Assessment Report. SEDAR, North Charleston, SC.

Smart, T.I., Reichert, M.J.M, Ballenger, J.C., Bubley, W.J., and Wyanski, D.M. 2015 Overview of sampling gears and standard protocols used by the Southeast Reef Fish Survey and its partners. SEDAR41-RD58. SEDAR, North Charleston, SC. 16 pp.

Takahashi, H. 1977. Juvenile hermaphroditism in the zebrafish, *Brachydanio rerio*. Bull. Fac. Fish.

Hokkaido Univ. 28:57-65.

Takahashi, H. AND M. Shimizu. 1983. Juvenile intersexuality in a cyprinid fish, the sumatra barb, *Barbus tetrazona tetrazona*. Ibid. 34:69-78.

Yamamoto, T. 1969. Sex differentiation, p. 117-175. In: Fish physiology III. W. S. Hoar and D. J. Randall (eds.). Academic Press, New York, New York.