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Scamp grouper reproduction in the Gulf of Mexico

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Introduction:

Scamp grouper, *Mycteroperca phenax*, are distributed along the western Atlantic and throughout the Gulf of Mexico (Hoese and Moore 1977) and support important recreational and commercial fisheries (Lombardi-Carlson et al., 2012). However, stock status in the Gulf of Mexico (GOM) is currently unknown. Scamp grouper are sequential protogynous hermaphrodites, in which males recruit from females and this can present unique challenges to both traditional stock assessments (Alonzo & Mangel 2005, Brooks et al. 2008, Shepherd et al. 2013) and spatial management (Easter & White 2016) because males recruit from females. Stock assessment models typically aggregate outputs across the spatial domain of the species (Berger et al. 2017) and assume reproductive success is female-driven (Easter & White 2016). Both of these assumptions can be erroneous in protogynous species (Brooks et al. 2008, Shepherd et al. 2013; Lowerre-Barbieri et al., 2020). Because of this, in addition to estimating female reproductive potential it is important to estimate sex ratios and the potential for sperm limitation.

Scamp grouper are a moderately long-lived species with a maximum age in the Gulf of Mexico of 31 years (Lombardi-Carlson et al., 2012). They are reported to have an extended spawning season from January through June, with a peak in April and to mature by age 2 (Lombardi-Carlson et al., 2012). In this report we developed species-specific histological indicators to assess maturity, reproductive timing, transition, and reproductive phases, estimated spawning seasonality, sex ratios, size and age at maturity and transition and spawning frequency.

Survey Design, Sampling Methods, and Analyses:

Most samples came from NMFS (n=4,105), collected from 1972 to 2017 from fishery-dependent, fishery-independent and unknown sources. Additional samples came from FWC from 2009 to 2017 (n=459), with more samples from 2018-2020 which are not included here. The FWC samples came from fishery-independent monitoring surveys (FIM), fishery-dependent monitoring surveys (FDM) and by-catch from a study targeting Gag Grouper along the western coast of Florida and have not yet been aged.

FWC samples of gonad tissue were collected and immediately fixed in 10% phosphate-buffered formalin. For histological analysis, ovarian tissue was fixed in 10% neutrally buffered formalin for 24 h, soaked in water for 1-2 h, and stored in 70% ethanol. Samples were embedded in glycol methacrylate, sectioned to 3–5- μm thickness, stained with periodic acid–Schiff's hematoxylin, and then counterstained with metanil yellow (Quintero-Hunter et al. 1991). Samples from NMFS were collected and preserved in 10% buffered formalin on board of scientific vessels, in ports where the fish were intercepted, or at the laboratory soon after the gonad tissue was removed from the fish. The gonad tissue remained in storage in 10% buffered formalin until time of processing. Trimmed subsamples were sent in histology cassettes to specialized laboratories for histological processing. The histological samples were then embedded in paraffin, sectioned to 4-6 μm in thickness, and stained using hematoxylin and eosin. Batch fecundity estimates (BFEs) were completed for 26 samples using the hydrated oocyte method from Hunter et al. (1985). Gonadal tissue was histologically assessed for all samples and sex and reproductive phases assigned. Because of the nature of sampling gonads from fishery-dependent sources, weights

were not always available. But for fish with gonad weights (n=1,279) the gonadosomatic index (GSI) was calculated as:

$$GSI = 100 * \left(\frac{gonad\ weight}{total\ weight - gonad\ weight}\right)$$

Gonadal analysis

Reproductive state, phase and histological indicators of Scamp were assigned following Lowerre-Barbieri et al. (2009) and Brown-Peterson et al. (2011); criteria are outlined in Table 1. Histological indicators for female Scamp reproductive phases and histological criterion are outlined in Table 2 and included: (1) oocyte developmental stages: primary growth (PG), cortical alveoli (CA), vitellogenic (Vtg1-3), and oocyte maturation (OM); (2) post ovulatory follicles (POFs); and (3) atresia. Secondary growth oocytes (SG) included CA, Vtg, and OM and fish with this level of development are considered to have received the physiological cue to develop oocytes for the coming spawning season (Lowerre-Barbieri et al., 2011b). However, gonadal development does not always correspond to functional maturity. There is no definitive histological indicator to distinguish immature from mature regenerating females, which both have only PG oocytes. However, because maturity is a process it is possible to use histological appearance of other aspects of the gonad to distinguish young immature females from old regenerating females. These include: a clearly defined lumen, the density and organization of the PG population, thickness of the ovarian wall, presence of capillaries and sometimes the occurrence of muscle bundles extending from the ovarian wall into the ovarian lamellae—but this last criterion is often difficult to use in groupers (Lowerre-Barbieri et al., 2011b; Lowerre-Barbieri et al. 2015). However, this level of histological detail was not always available for historical samples. Thus, for size and age at maturity estimates we used only spawning capable and immature, but immature females were excluded from the historical data due to known issues distinguishing between immature and regenerating individuals. The Panama City group is currently reanalyzing their historical slides with the above criteria to evaluate what is needed to standardize assignments throughout their data set.

Oocyte maturation was broken down into sub-stages: germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), yolk coalescence or clarification, and oocyte hydration (Jalabert 2005). Postovulatory follicles (POFs) were classified as either newly collapsed (recognizable by the size and appearance of the granulosa cells' nuclei) or 12 h or older based on POF size, organization, and elapsed time from peak spawning (Hunter & Macewicz 1985). Actively spawning females were considered to be those undergoing late OM, ovulation, or with fresh POFs (Tables 1 and 2). The duration of both OM and POFs in Scamp was considered to be 48 h.

Scamp are not dimorphic. Because Scamp transition from female to male, testes continue to have ovarian walls and often large numbers of primary growth oocytes. Because of this, histological analysis is needed to assign sex. Fish were considered male if only spermatogenic cells were present (i.e., no PG) or they had spermatozoa present (Trip et al., 2011). Similarly, sex was determined as female if there was nothing but female tissue or healthy SG oocytes were present. Parasitic nematodes were frequently observed in histological slides. Small cross sections of parasites looked similar to yolked oocytes undergoing atresia, with the exception of an external

epithelial layer (Figure 5, right). Parasites occurred in both ovaries and testes and immature and mature females and had previously been considered signs of previous spawning and thus maturity in the NMFS samples.

We defined fish undergoing sex change as transitional (no sex assigned) and broke this down into early and late transition. Early transition is defined as those fish with spermatagonia, spermatocytes, and some spermatids. Late transition includes proliferating amounts of male tissue with spermatids or later stages of spermatogenesis present (Table 3). Estimates of size and age at transition were based on all females and male but did not include transitionals.

Maturity and transition

Binomial generalized linear models (GLMs) were used to model maturity and transition at age and length. Different link functions (logit, probit, clogloc and cauchit) were specified, and the best model was chosen via Akaike Information Criterion (AIC). Models were fitted in R and model comparison was performed using the R package 'MuMIn'. Estimated parameters were the intercept and slope. The inflection point (age or length at 50% maturity or transition) was calculated by dividing the absolute value of model intercept by slope. To decrease the uncertainty associated with assigning maturity, we selected for samples collected during the spawning season and used only spawning capable females to represent mature females. This had the effect of decreasing the numbers of regenerating females which are difficult to unambiguously identify, while spawning capable females are the easiest to unambiguously identify and also the closest to spawning.

Spawning frequency

There is no standardized method to assign spawning season, and age-specific differences in spawning period duration can have a large impact on estimates of annual fecundity (Lowerre-Barbieri et al., 2011b). Due to low numbers of aged samples, it was not possible to estimate age-specific spawning seasons. To assess the total population duration of spawning activity, we defined the time period between the first and last dates that female active spawners were observed. However, due to spawning activity being asynchronous and not evenly distributed over this time period (Figure 3D), we estimated the core spawning season (i.e., 50% or more of the females were spawning capable) using a binomial regression to model calendar date and spawning state data. We selected spawning capable and developing females to determine the mid-point for the beginning of the spawning season and spawning capable and regressing females to estimate when > 50% of females were no longer spawning capable.

Actively spawning females were identified as those with indicators of imminent or recent spawning (i.e., OM or POFs). Spawning fraction was estimated as the proportion of all females which were actively spawning. This follows Porch et al. 2015 and is a modification of the traditional Hunter and Macewicz (1985) methodology, in that spawning indicators were combined (OM and POFs) and the denominator included all females, regardless of maturity. When estimating total egg production this is considered a better measure of reproductive potential and removes the potential uncertainty with maturity assignments (Porch, pers.comm.). Spawning interval was estimated as the reciprocal of spawning fraction divided by the number of days that indicators were identifiable (i.e., for scamp 48 h or 2 D). Spawning frequency was estimated as the number of days in the population spawning season divided by the spawning

interval. Polynomial and logistic regression models were fitted to the spawning frequency at age models, and the best fitting model was selected using AIC. The logistic model that was fitted was as follows:

$$y = \frac{\kappa}{(1 + (e^{-a_1*(x-a_0)})}$$

where x was age in years, κ was the spawning frequency asymptote, and a_1 and a_0 were slope and intercept, respectively, of the ascending limb. The model was fitted using maximum likelihood, and the variance parameter of the likelihood function was an additional estimated parameter.

Batch fecundity
Batch fecundity (BF) typically increases with length, somatic weight, and age. However, sample size was too small to assess the BF with somatic weight (n=5) and age (n=9) relationships. Although residuals from a simple linear regression did not suggest a curvilinear relationship, $\log_e$ transforming the variables slightly improved the adjusted R^2. Linear regression was run on these $\log_e$-transformed parameters.

Best measure of reproductive potential:
Reproductive success is accomplished through trade-offs between the rate of reproductive output and the survivorship rate associated with that output. To integrate the concept of reproductive success into stock assessment processes, Trippel (1999) introduced the term "stock reproductive potential," defined as the "annual variation in a stock's ability to produce viable eggs and larvae that may eventually recruit to the adult population or fishery." Traditionally, it has been assumed that reproductive success in marine fishes is primarily driven by fecundity. Although reproductive success is tightly coupled with adult abundance and fecundity in many terrestrial animals, it is less so in marine fishes which have extreme adult to offspring size ratios, offspring mobility and mortality. Spawner-recruit systems in marine fishes are species-specific with traits occurring over spatial, temporal and biological scales and thus there is not one measure of reproductive potential which is accurate for all species. Thus, the best measure will depend on data availability, spatio-temporal reproductive behavior, demographic drivers of reproductive value, and gender system (i.e., sequential hermaphrodite or gonochoristic; Lowerre-Barbieri et al., 2017). Given that Scamp are protogynous, it is important to consider the contribution of males, as well as females to reproductive potential (Brooks et al., 2008; SEDAR 2015; Figure 1).

Results / Discussion:

A total of 4,564 Scamp were sampled from 1972 to 2017 in the Eastern Gulf of Mexico and had histological analysis of gonadal development (Table 4). Those from 2002 and earlier were analyzed in Lombardi et al. (n=2,634). New, previously unanalyzed samples (n=1,930) have been collected from 2003-2017 (Figure 2). In this new period 459 samples came from FWC and 1,471 from NMFS. All of these samples had gonadal tissue examined histologically to assign reproductive phase and sex.

Of all fish sampled, there were: 1,675 males, 2,754 females and 135 transitionals. The earlier study period had a male sex ratio of 36% (914 males, 1,638 females, and 82 transtionals) compared to 41% in more recent sampling (761 males, 1,116 females and 53 transitionals). Although male, female, and transitional sizes overlapped (Figure 3) mean size by sex differed. The mean size of females was 469.6 +/- 88.9 mm FL compared to 578.7 +/- 72.7 mm FL for males and 499.8 +/- 78.2 for transitionals. Changes in size with sex by study period were minimal and differences in sex-specific sizes were significant (Kruskal-Wallis, $\chi 2=1399.9727$, $P < 0.0001$). In the 1970's the male sex ratio was estimated at 37.9%, with a decrease to 18-24% in the 1990's (Coleman et al., 1996) and has how increased to 41%.

Spawning seasonality

Females with spawning indicators were first sampled on 2 February and last sampled on 25 July (spawning season duration=173 d). However, most spawning capable females (88%) were collected in the months of March, April and May. Actively spawning females and female GSI peaked in April (Figure 4). Using a binomial regression to estimate the time period over which 50% or more of mature females are spawning capable resulted in roughly equal fits with the complementary-log-log (cloglog) and probit link functions. The ΔAIC value between the two models was 0.62 for the start of the spawning season and 5.97 for the end of the spawning season, with the cloglog model performing slightly better in both cases. Under the cloglog link, the estimated spawning season was March 9th through May 26th (79 d), and under the probit fit it was February 28 through June 7 (100 d). Atlhough the cloglog link had slightly better performance, we consider the probit model estimates to be more realistic as they better capture the time period when the majority of active spawning was observed (Figure 4 C and D).

Age and size at maturity

Estimated parameters with uncertainty estimates for length and age at maturity and transition are shown in table 5. The logit link function provided the best fit to maturity at age and transition at length data, and the probit link was the best fitting model for maturity at length and transition at age. Within the spawning season of 2 February to 25 July, 763 females had good quality histology slides and were either immature or spawning capable. Four hundred and thirteen of these had assigned ages. Immature females ranged in ages from 1 to 7, and the youngest mature female was age 3 (Table 6; Figure 5). Estimated age at 50% mature was 3.4 years (Figure 6). Immature females ranged in size from 178 to 438 mm FL, with a mean of 357.3 mm FL (Table 7). The size of spawning capable females ranged from 281 to 740 mm FL, with a mean of 512 mm FL. Estimated size at 50% maturity was 363.7 mm FL (Figure 6).

Age and size at transition

The youngest observed males were three years old (Table 8) and the smallest observed male was 221 mm (Table 9; there was no age for this individual). Estimated age at 50% male was 10.8 y (Figure 7). Transitionals (n=136) ranged in size from 299 to 710 mm FL, with a mean size of 499.8 mm FL. The temporal distribution of transitionals was extensive, with samples collected in every month of the year. Estimated size at 50% male was 555 mm (Figure 7).

Spawning frequency

Only 751 females sampled during the spawning season have been aged. Estimated spawning fraction was zero for ages 1 and 2, then increased for ages 3 and 4, and started plateauing at age

5. The largest spawning fraction was observed for age six (shortest spawning interval of 4.44 days) which was also the age group with the largest available sample size (n_{age6} = 100). After age 12, available samples decreased to fewer than 20, and ages 14 to 19 were pooled due to low sample size. Thus, it is not possible to confirm that the declining apparent spawning fraction with age was not affected by lower sample sizes.

The estimate of spawning season length has a large impact on spawning frequency estimates. Since there is some uncertainty about the best estimate of spawning season length, we calculated spawning frequency for all three estimates (79, 100, and 173 days), with the middle length of 100 days being the base estimate. Regardless of spawning season length, the logistic model provided the best fit to the observed spawning frequency at age data (AIC = 74.41; 100 day ss) followed by the second order polynomial model (AIC = 79.8; 100 day ss) and the third order polynomial model (AIC = 79.9). Spawning frequency under the logistic model plateaued at 16.5 days days per season if ss length of 100 days is used (Figure 8), 28.5 days per season if ss length is 173 days, and 13 days if the spawning season is 79 days (Table 10).

Batch fecundity
There was not sufficient data from the Gulf (n=9) to develop a robust batch fecundity to size relationship.

Measure of reproductive potential
Because Scamp do not exhibit a 1:1 sex ratio and there are significant differences between size and age at sex, we recommend integrating male contributions to reproductive potential. Because there is not a male equivalent of total egg production, we recommend using combined biomass but exploring the most ecologically realistic measures of demographic and sex-specific reproductive value.

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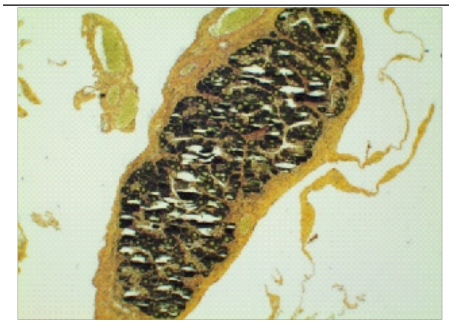
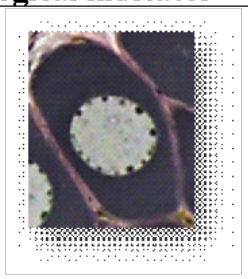
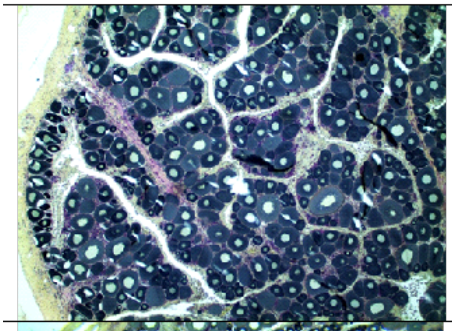
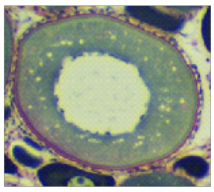
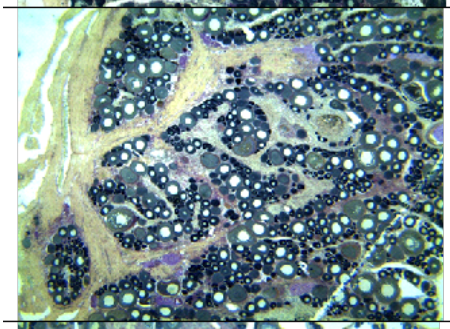
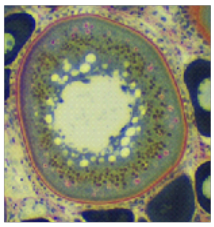
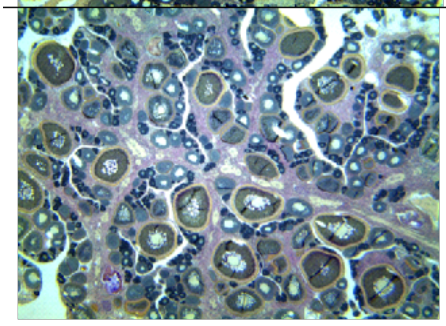
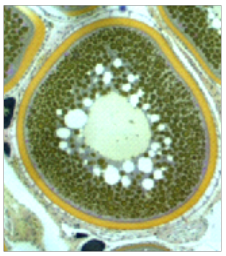
Table 1. Ovarian classification and terms based on histological analysis (modified from Lowerre-Barbieri et al., 2009).

Reproductive state	Phase	Histological indicators	Significance
Non-spawning	Immature	Only oogonia and primary growth oocytes, including chromatin nucleolar and perinucleolar oocytes. Usually no atresia.	Virgin that has not yet recruited to the spawning population.
Non-spawning	Developing	Cortical alveolar and sometimes early yolked oocytes. No evidence of POFs. Some atresia may be present.	Mature or maturing. Environmental signals have triggered the maturation process, but fish are not yet developed enough to spawn.
Spawning	Spawning-capable	Yolked oocytes. May have some early OM and/or some atresia; fish which have spawned within the past 48 h may have remnant POFs	Part of the spawning population. Fish developed enough to spawn.
Spawning	Sub-phase: Actively Spawning	Late OM (completed GVM or GVBD with yolk coalescence and partial to full hydration); ovulation; or newly-collapsed POFs	Part of the spawning population. Fish sampled in close proximity to the time of spawning and thus useful for assessing spawning sites.
Non-spawning	Regressing	A high percentage of yolked oocytes undergoing atresia (alpha and beta).	Mature fish at the end of the spawning season, resorbing left over developed oocytes.
Non-spawning	Regenerating	Only primary growth oocytes present, including chromatin nucleolar and perinucleolar. Muscle bundles, enlarged blood vessels, thick and/ or convoluted ovarian wall, and gamma or delta atresia may be present.	Sexually mature, reproductively inactive. Most common outside of the spawning season.

Table 2. Histological basis for reproductive phases in female Scamp, *Mycteroperca phenax*

Ovarian Cross Section	Phase Characteristics	Most advanced oocyte or key histological indicator
	**Immature Phase** • Only oogonia & PG • No muscle bundles • Thin ovarian wall • Small ovaries • Organized lamellae	 Perinucleolar primary growth (PG)
	***Early Developing – Sub phase*** • PG & CA • Can be some atresia	 Cortical alveolar (CA)
	**Developing** • PG & CA, Vtg1 (partially yolked) • No Vtg3 or POFs • Can be some atresia	 Vtg1 partially yolked
	**Spawning Capable** • Vtg3 (fully yolked oocytes FY) present • Can have early oocyte maturation (OM) • Can be some atresia	 Vitellogenic 3

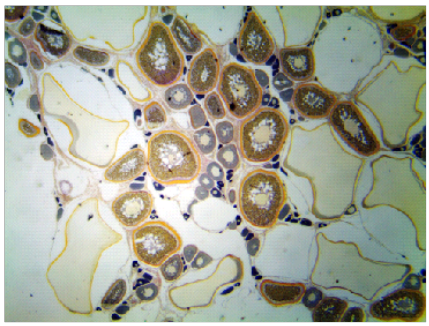
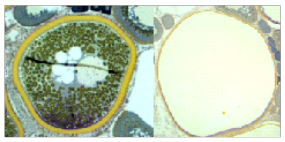
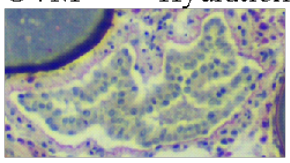
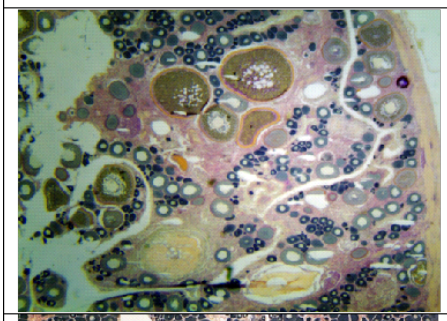
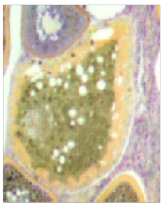
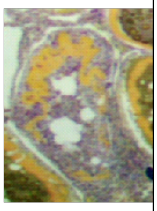
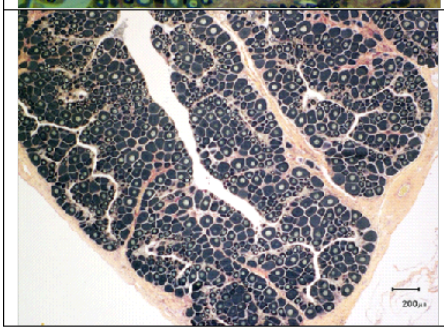
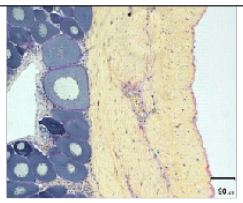
	***Actively Spawning subphase*** • Late germinal vesicle migration (GVM) • Germinal vesicle breakdown (GVBD) • Hydration and can have fresh POFs	 GVM Hydration  Fresh POF
	**Regressing** • Most Vtg oocytes undergoing atresia	  Alpha atresia Beta atresia
	**Regenerating** • Oogonia and PG oocytes present • Muscle bundles • Thick ovarian wall	 Thick ovarian wall

Table 3. Histological indicators of fish undergoing transition in male Scamp, *Mycteroperca phenax morio.*

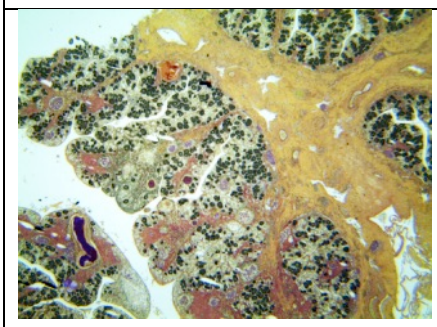
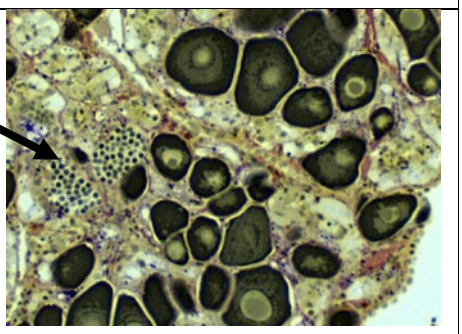
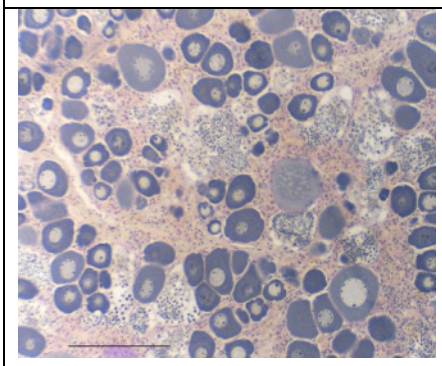
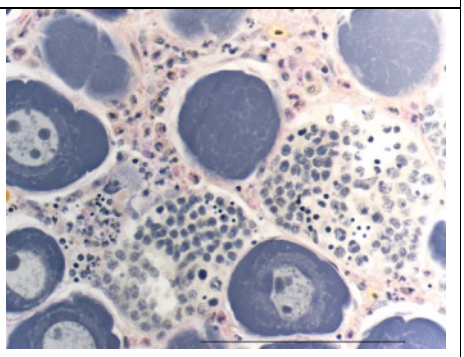
Teste Cross Section	Phase Characteristics	Key histological indicator
	**<u>Early transition</u>** • Spermatagonia (Sg) & spermatocytes (Sc) present • Continuous germinal epithelium • PG abundance decreasing	
	**<u>Mid-late transition</u>** • Sg, Sc & spermatids (St) present, occasional sperm crypts • Male tissue proliferation is dominant	

Table 4. Sample availability for scamp reproductive analysis by agency that collected the data (NMFS = National Marine Fisheries Service, FWC = Florida Fish and Wildlife Conservation Commission) and sample source (COM = Commercial, REC = recreational, FIM = Fisheries-independent Monitoring, RES = Research, UNK = unknown.

Year	COM			REC			FIM			RES			UNK			Grand Total
	NMFS	FWC	Total	NMFS	FWC	Total	NMFS	FWC	Total	NMFS	FWC	Total	NMFS	FWC	Total	
1972	0	0	0	0	0	0	0	0	0	0	0	0	7	0	7	7
1973	0	0	0	0	0	0	0	0	0	0	0	0	15	0	15	15
1977	28	0	28	0	0	0	1	0	1	0	0	0	20	0	20	49
1978	230	0	230	7	0	7	17	0	17	0	0	0	123	0	123	377
1979	312	0	312	0	0	0	29	0	29	0	0	0	97	0	97	438
1980	142	0	142	0	0	0	23	0	23	0	0	0	21	0	21	186
1982	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	4
1991	159	0	159	0	0	0	12	0	12	0	0	0	0	0	0	171
1992	5	0	5	0	0	0	16	0	16	0	0	0	0	0	0	21
1993	133	0	133	2	0	2	26	0	26	0	0	0	0	0	0	161
1994	88	0	88	5	0	5	85	0	85	0	0	0	3	0	3	181
1995	105	0	105	2	0	2	51	0	51	0	0	0	0	0	0	158
1996	44	0	44	0	0	0	95	0	95	0	0	0	0	0	0	139
1997	4	0	4	14	0	14	30	0	30	0	0	0	0	0	0	48
1998	12	0	12	1	0	1	43	0	43	0	0	0	0	0	0	56
1999	28	0	28	30	0	30	49	0	49	0	0	0	0	0	0	107
2000	33	0	33	28	0	28	9	0	9	0	0	0	0	0	0	70
2001	54	0	54	10	0	10	2	0	2	0	0	0	0	0	0	66
2002	276	0	276	30	0	30	73	0	73	0	0	0	1	0	1	380
2003	312	0	312	23	0	23	55	0	55	0	0	0	0	0	0	390
2004	166	0	166	18	0	18	4	0	4	0	0	0	0	0	0	188
2005	191	0	191	14	0	14	5	0	5	0	0	0	0	0	0	210
2006	11	0	11	0	0	0	3	0	3	0	0	0	0	0	0	14

2007	8	0	8	18	0	18	0	0	0	0	0	0	0	0	0	26
2008	16	0	16	7	0	7	0	0	0	0	0	0	0	0	0	23
2009	12	0	12	14	12	26	15	0	15	0	0	0	0	0	0	53
2010	0	0	0	0	20	20	2	0	2	0	0	0	0	0	0	22
2011	10	0	10	5	10	15	0	0	0	0	0	0	0	0	0	25
2012	152	0	152	5	0	5	0	0	0	0	0	0	0	0	0	157
2013	166	0	166	9	129	138	16	0	16	0	0	0	1	0	1	321
2014	24	0	24	6	19	25	36	0	36	0	0	0	0	0	0	85
2015	19	0	19	6	43	49	31	0	31	0	0	0	0	0	0	99
2016	8	0	8	9	46	55	12	0	12	0	11	11	0	0	0	86
2017	0	83	83	7	31	38	55	0	55	0	55	55	0	0	0	231
Grand Total	2748	83	2831	270	310	580	795	0	795	0	66	66	292	0	292	4564

Table 5. Parameter estimates for scamp maturity and transition regression models. Parameter values for the best fitting model and the logit link are provided.

Model	Link Fct	Mod_weight	N	Parameter	Estimate	Std Error
F_mat_age	logit	0.945	413	Intercept	-4.55E+00	7.31E-01
				slope	1.33E+00	1.79E-01
F_mat_length	probit	0.465	763	Intercept	-7.90E+00	8.50E-01
				slope	2.17E-02	2.13E-03
	logit	0.368		Intercept	-1.47E+01	1.66E+00
				slope	4.03E-02	4.20E-03
Transition_age	probit	0.888	1,937	Intercept	-2.15E+00	9.48E-02
				slope	1.99E-01	9.81E-03
	logit	0.112		Intercept	-3.59E+00	1.74E-01
				slope	3.33E-01	1.77E-02
Transition_length	logit	1	4,412	Intercept	-9.48E+00	3.05E-01
				slope	1.71E-02	5.65E-04

Table 6. Observed and predicted proportion mature at age.

Age (years)	*N*	Observed proportion mature	Predicted proportion mature (logit fit)
0	0	NA	0.02
1	1	0.00	0.07
2	12	0.00	0.23
3	36	0.42	0.53
4	43	0.67	0.81
5	47	0.94	0.94
6	66	0.96	0.98
7	40	0.98	1.00
8	35	1.00	1.00
9	40	1.00	1.00
10	33	1.00	1.00
11	26	1.00	1.00
12	15	1.00	1.00
13	8	1.00	1.00
14	8	1.00	1.00
15	1	1.00	1.00
16	0	NA	1.00
17	1	1.00	1.00

Table 7. Observed and predicted proportion mature at length.

Fork length (mm)	*N*	Observed prop. mature	Predicted prop. mature (probit fit)	Pred prop. mature (logit fit)
100-249	9	0.00	0.00	7.62E-03
250	2	0.00	0.01	1.14E-02
260	3	0.00	0.02	1.69E-02
270	0	NA	0.03	2.51E-02
280	4	0.25	0.04	3.70E-02
290	2	0.00	0.07	5.44E-02
300	3	0.00	0.10	7.93E-02
310	6	0.50	0.15	1.14E-01
320	2	0.00	0.20	1.62E-01
330	5	0.40	0.27	2.24E-01
340	7	0.29	0.34	3.01E-01
350	12	0.33	0.43	3.92E-01
360	12	0.58	0.51	4.91E-01
370	20	0.55	0.60	5.91E-01
380	31	0.55	0.68	6.84E-01
390	26	0.73	0.75	7.64E-01
400	21	0.81	0.82	8.29E-01
410	23	0.91	0.87	8.79E-01
420	28	0.93	0.91	9.15E-01
430	37	0.95	0.94	9.42E-01
440	36	1.00	0.96	9.60E-01
450	34	1.00	0.98	9.73E-01
460	37	1.00	0.99	9.82E-01
470	26	1.00	0.99	9.88E-01
480	47	0.98	1E+00	9.92E-01
490	32	1.00	1E+00	9.95E-01
500	37	1.00	1E+00	9.96E-01
510	47	1.00	1E+00	9.98E-01
520	28	1.00	1E+00	9.98E-01
530	28	1.00	1E+00	9.99E-01
540	17	1.00	1E+00	9.99E-01
550	21	1.00	1E+00	1.00E+00
560-599	64	4.00	1E+00	1.00E+00
600-699	48	9.00	1E+00	1.00E+00
700-799	6	4.00	1E+00	1.00E+00
800-839	2	2.00	1E+00	1.00E+00

Table 8. Observed and predicted proportion male at age.

Age (years)	*N*	Observed proportion male	Predicted proportion male (probit fit)	Predicted proportion male (logit fit)
1	4	0.00	0.03	0.04
2	41	0.00	0.05	0.06
3	124	0.02	0.07	0.08
4	121	0.06	0.11	0.11
5	168	0.12	0.15	0.15
6	201	0.21	0.20	0.20
7	142	0.24	0.26	0.25
8	176	0.34	0.32	0.32
9	188	0.38	0.40	0.40
10	192	0.45	0.48	0.48
11	153	0.47	0.56	0.56
12	140	0.62	0.63	0.64
13	89	0.64	0.71	0.71
14	63	0.71	0.77	0.78
15	40	0.83	0.83	0.83
16	30	0.97	0.87	0.87
17	22	0.68	0.91	0.90
18	11	0.91	0.94	0.93
19	7	0.71	0.96	0.95
20	6	1.00	0.97	0.96
21	8	1.00	0.98	0.97
22	3	1.00	0.99	0.98
23	3	1.00	0.99	0.99
24	1	1.00	1.00	0.99
25	1	1.00	1.00	0.99
26	0	NA	1.00	1.00
27	0	NA	1.00	1.00
28	1	1.00	1.00	1.00
29	1	1.00	1.00	1.00
30	1	1.00	1.00	1.00

Table 9. Observed and predicted proportion male at length.

Fork length (mm)	*N*	Observed proportion male	Predicted proportion male (logit fit
100-219	9	0.00	0.00
220	4	0.25	0.00
230	5	0.00	0.00
240	10	0.00	0.00
250	7	0.14	0.01
260	5	0.00	0.01
270	18	0.00	0.01
280	20	0.00	0.01
290	21	0.00	0.01
300	23	0.04	0.01
310	28	0.00	0.02
320	29	0.00	0.02
330	23	0.00	0.02
340	27	0.04	0.03
350	54	0.04	0.03
360	64	0.02	0.04
370	84	0.01	0.04
380	104	0.01	0.05
390	89	0.01	0.06
400	86	0.09	0.07
410	98	0.02	0.08
420	113	0.04	0.10
430	134	0.08	0.11
440	133	0.14	0.13
450	131	0.14	0.15

460	135	0.20	0.18
470	155	0.26	0.21
480	173	0.20	0.24
490	157	0.24	0.27
500	176	0.30	0.31
510	207	0.36	0.34
520	167	0.49	0.38
530	187	0.42	0.43
540	181	0.54	0.47
550	176	0.57	0.51
560	167	0.54	0.56
570	171	0.57	0.60
580	141	0.63	0.64
590	146	0.61	0.68
600	120	0.73	0.72
610	126	0.80	0.75
620	92	0.79	0.78
630	67	0.79	0.81
640	65	0.89	0.84
650	61	0.80	0.86
660	33	0.85	0.88
670	33	0.82	0.90
680	26	0.88	0.91
690	26	0.85	0.92
700	24	0.88	0.94
710	18	0.89	0.95
720	13	0.85	0.95
730	10	0.90	0.96
740	8	0.63	0.97

750	6	0.83	0.97
760	2	0.00	0.98
770	1	1.00	0.98
780	8	1.00	0.98
800	3	0.67	0.99
810	3	1.00	0.99
820	4	1.00	0.99
830	1	0.00	0.99
860	2	0.50	1.00
870	1	0.00	1.00

Table 10. Estimated spawning frequency and model fit. All females (mature and immature) were used to estimate spawning fraction. Results are shown for the base spawning season length estimate (100 days) and two alternative spawning season length estimates (79 days and 173 days).

Calendar age (yrs)	N spawning	N non-spawn	N total	Spawning fraction	Spawning interval	Spawning frequency	predicted (logit)	Spawning frequency	predicted (logit)	Spawning frequency	predicted (logit)
						100 day spawning season		*79 day spawning season*		*173 day spawning season*	
1	0	2	2	0		0	0.14		0.11		0.24
2	0	21	21	0		0	0.86		0.68		1.48
3	8	59	67	0.060	16.75	5.97	4.31	4.72	3.41	10.3	7.46
4	13	56	69	0.094	10.62	9.42	11.48	7.44	9.07	16.3	19.87
5	33	64	97	0.170	5.88	17.01	15.45	13.44	12.21	29.4	26.74
6	45	55	100	0.225	4.44	22.50	16.33	17.78	12.90	38.9	28.24
7	23	37	60	0.192	5.22	19.17	16.47	15.14	13.01	33.2	28.49
8	23	44	67	0.172	5.83	17.16	16.49	13.56	13.03	29.7	28.53
9	25	42	67	0.187	5.36	18.66	16.50	14.74	13.03	32.3	28.54
10	15	55	70	0.107	9.33	10.71	16.50	8.46	13.03	18.5	28.54
11	18	31	49	0.184	5.44	18.37	16.50	14.51	13.03	31.8	28.54
12	11	24	35	0.157	6.36	15.71	16.50	12.41	13.03	27.2	28.54
13	5	14	19	0.132	7.60	13.16	16.50	10.39	13.03	22.8	28.54
14	7	21	28	0.125	8.00	12.50	16.50	9.88	13.03	21.6	28.54

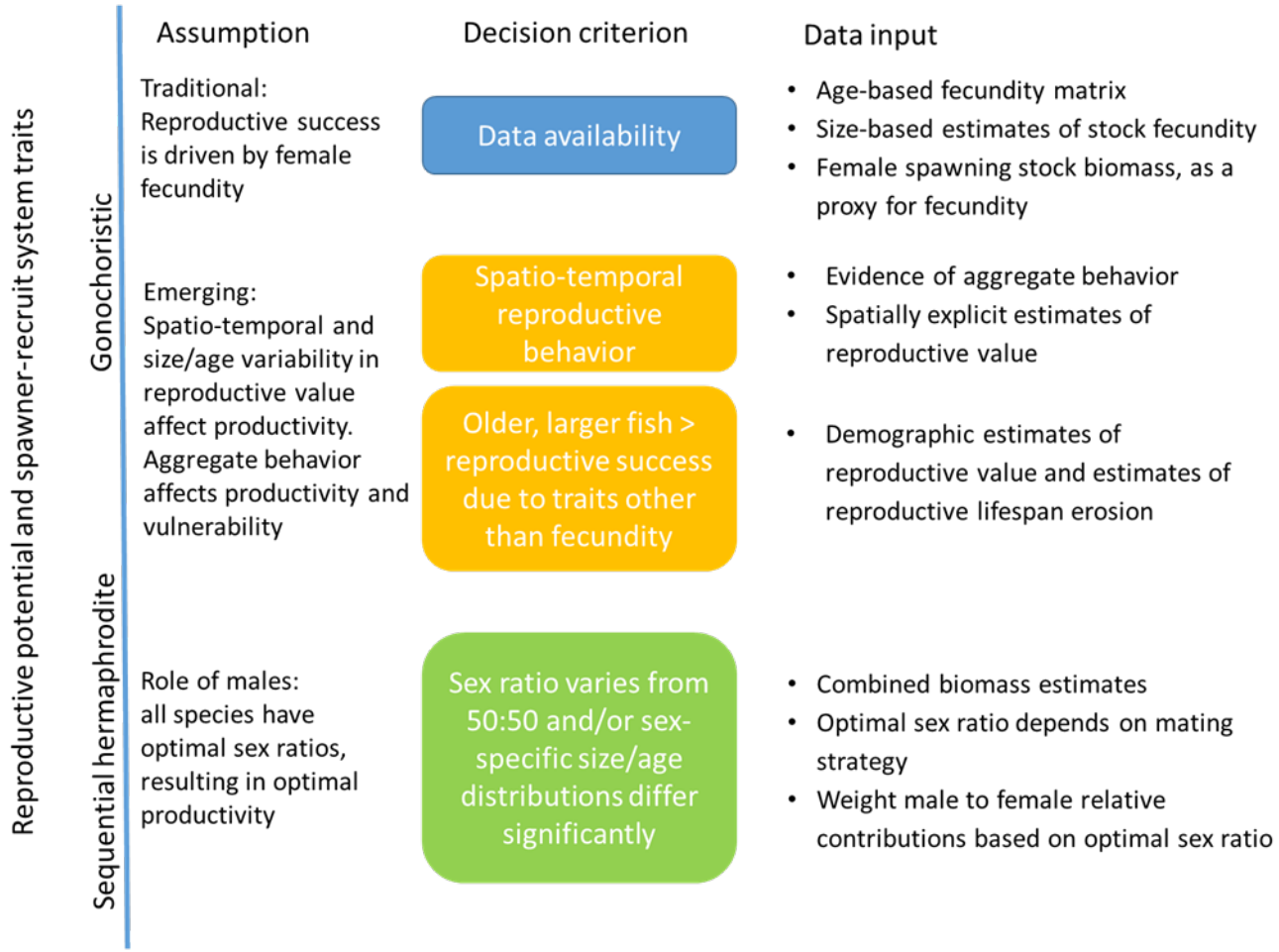


Figure 1. Modified from figure 6 in Lowerre-Barbieri et al., 2017: underlying assumptions associated with traditional and emerging understanding of reproductive potential, decision criterion and data needed.

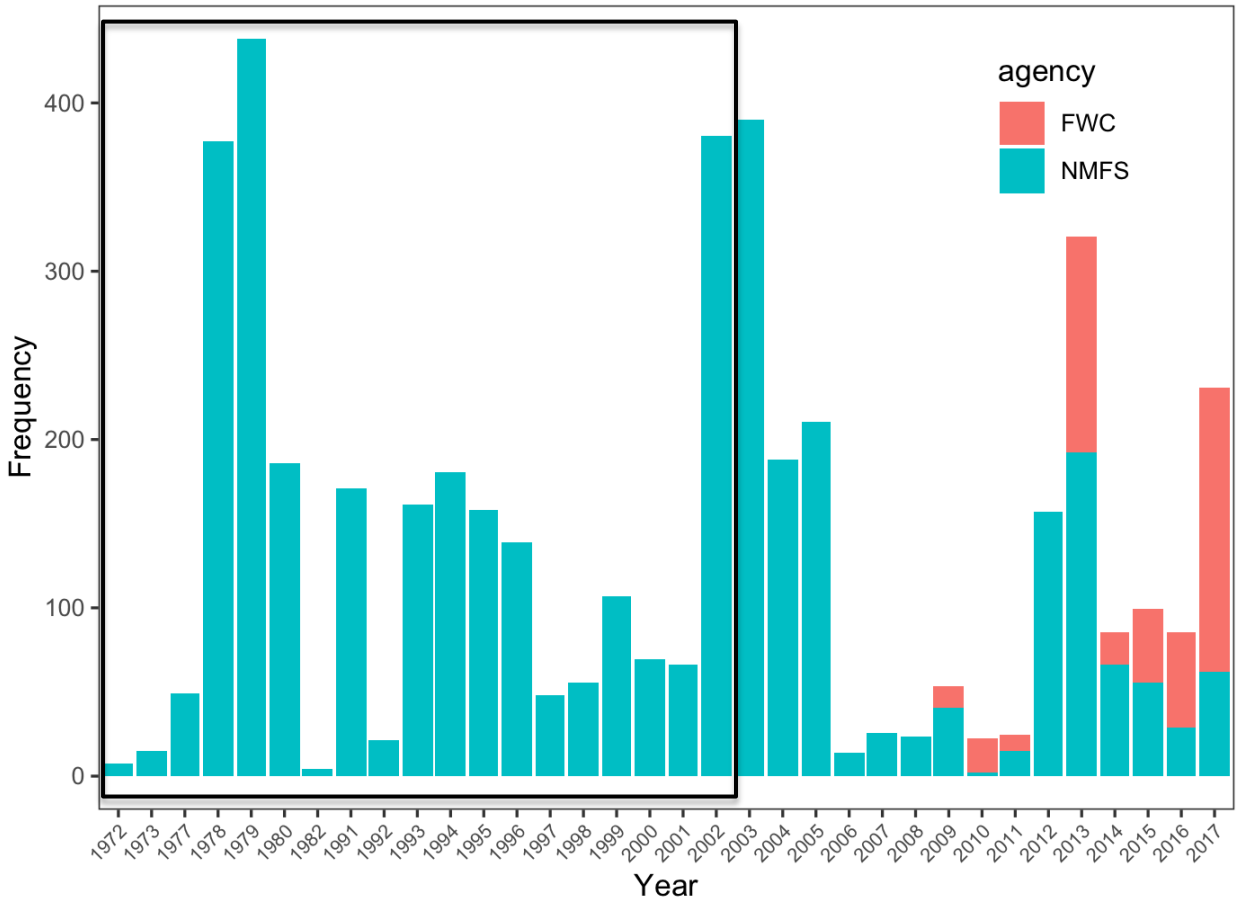


Figure 2. Sample size by year and agency which collected the data. The years within the rectangle were analyzed in Lombardi et al. 2012 and assigned to study period 1. More recent samples that have not been previously analyzed and published were assigned to study period 2.

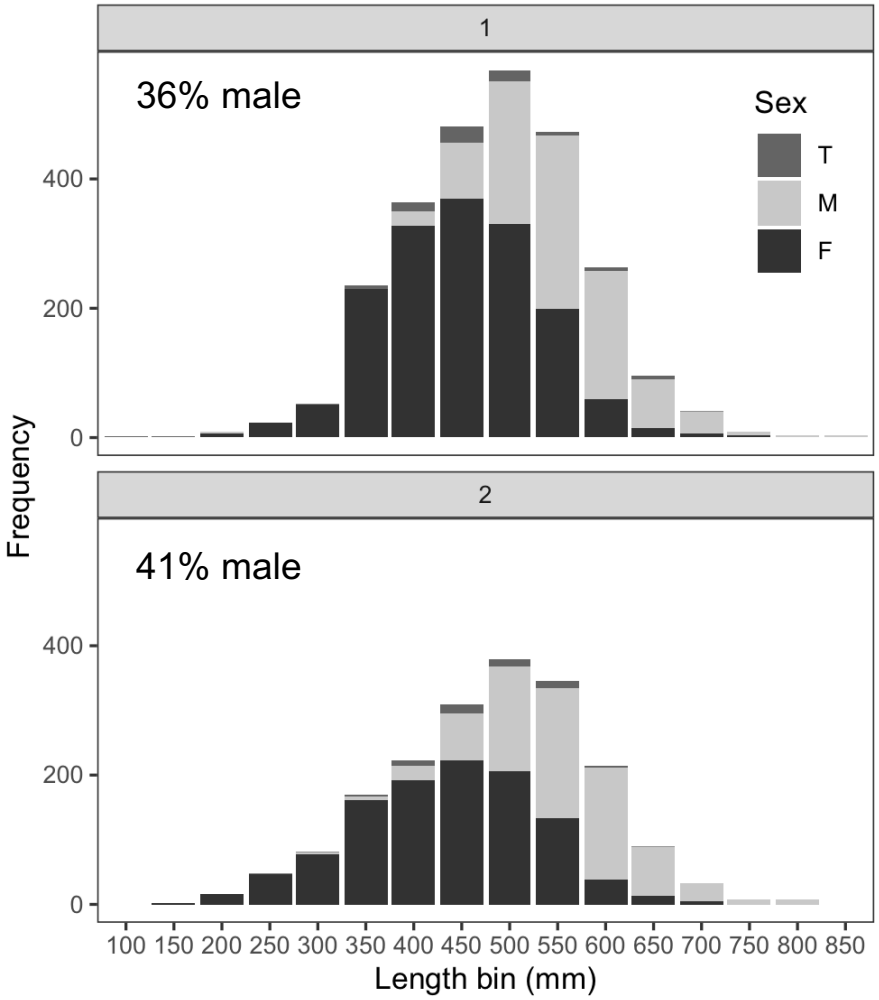


Figure 3. There was a lot of overlap in size between males and females, however males on average were larger. Transitionals were intermediate in size. The male sex ratio has increased in more recent years from 36% in the early period to 41%.

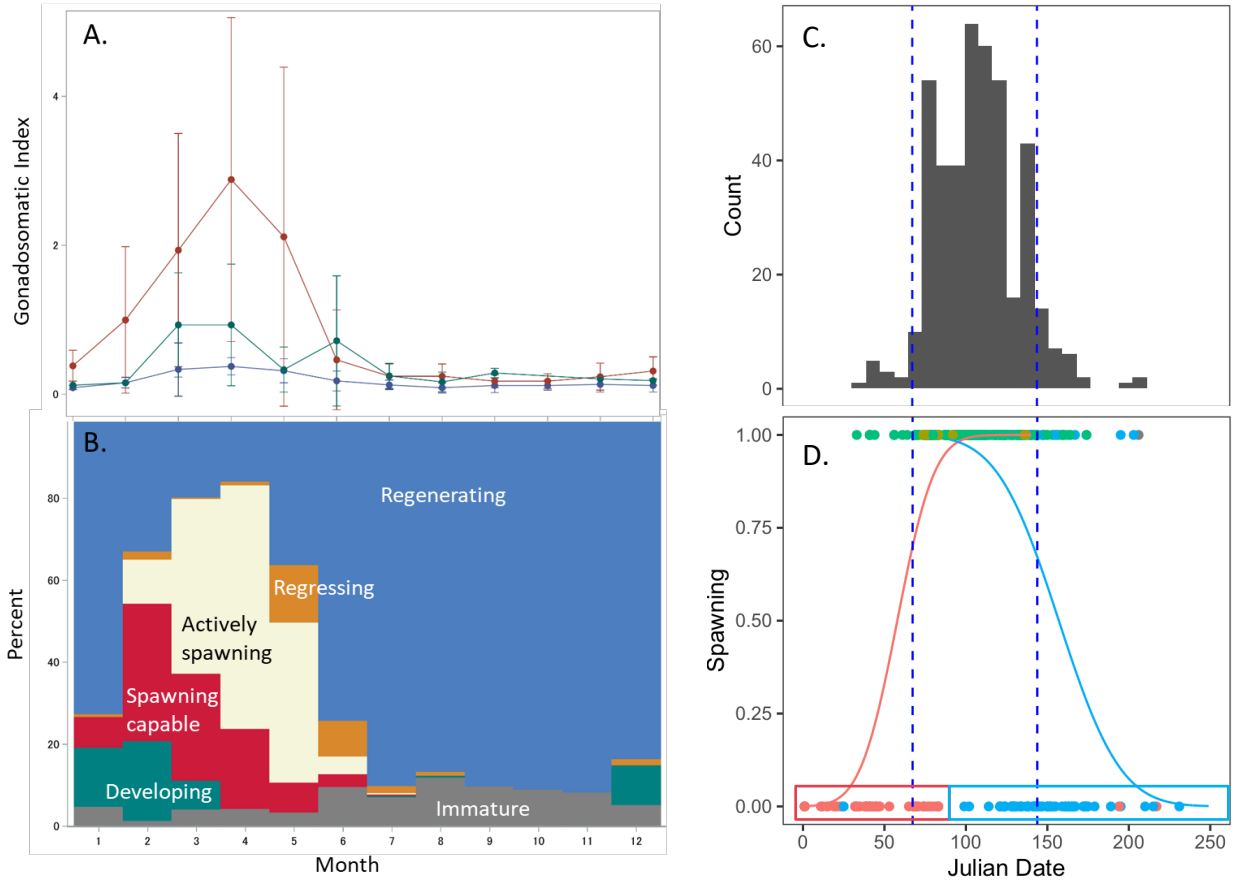


Figure 4. (A) Monthly mean gonadosomatic index +/- one standard deviation (red=female, green=transitional, blue=male and (B) monthly distribution of reproductive phases. (C) Although females with spawning indicators were collected from 2 February through 25 July, spawning activity was not equally distributed over this time frame and the best estimate of population spawning season was February 28 through June 7 (100 days; indicated by the dashed vertical lines) based on fitting a binomial regression (the probit link function fit is shown) to calendar date to estimate when 50% of the population was spawning capable. (D) the results from 2 separately fitted binomial regression models (one for spawning season start date and the other for the end date) in one graph. Colors in D correspond to assigned reproductive phases: red = developing, brown = spawning capable, green = actively spawning, blue = regressing, grey = NA. Spawning state (0 = non-spawning, 1 = spawning) was assigned based on the presence of hydrated oocytes or recent post-ovulatory follicles.

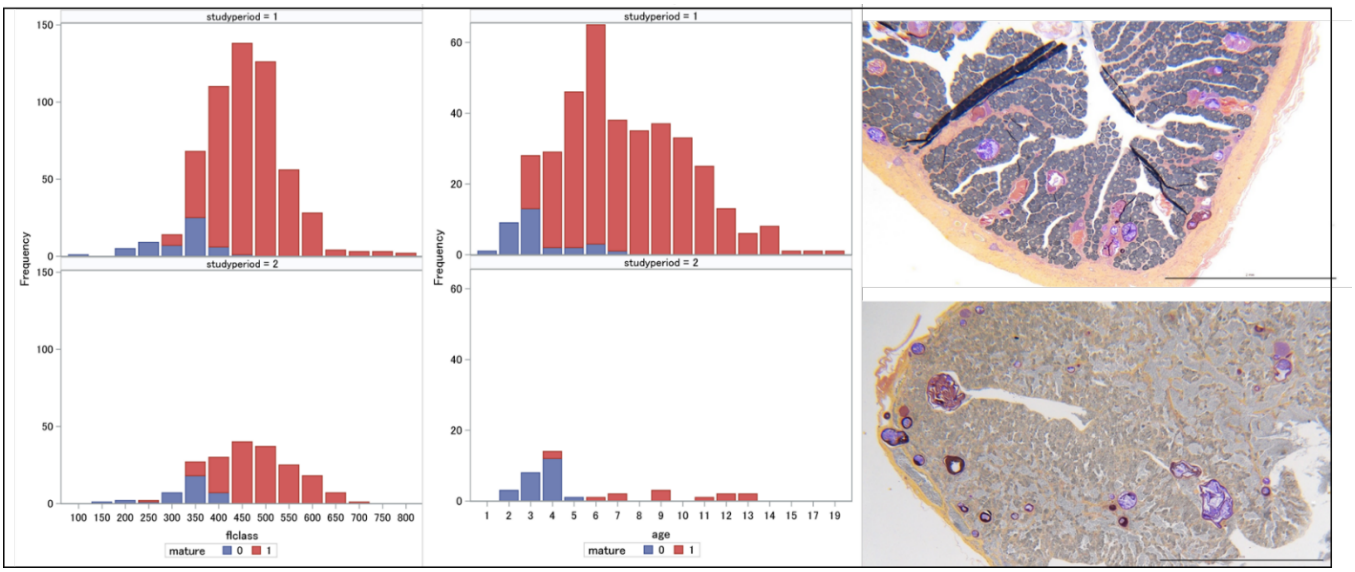


Figure 5. Frequency distributions of immature (blue) versus mature (red) females by study period for fork length (left) and age (middle). Mature females were based only on those which were spawning capable or immature. 0 = Immature, 1 = Mature. Previously parasites (right) have been mistakenly used as indicators of previous spawning and thus maturity.

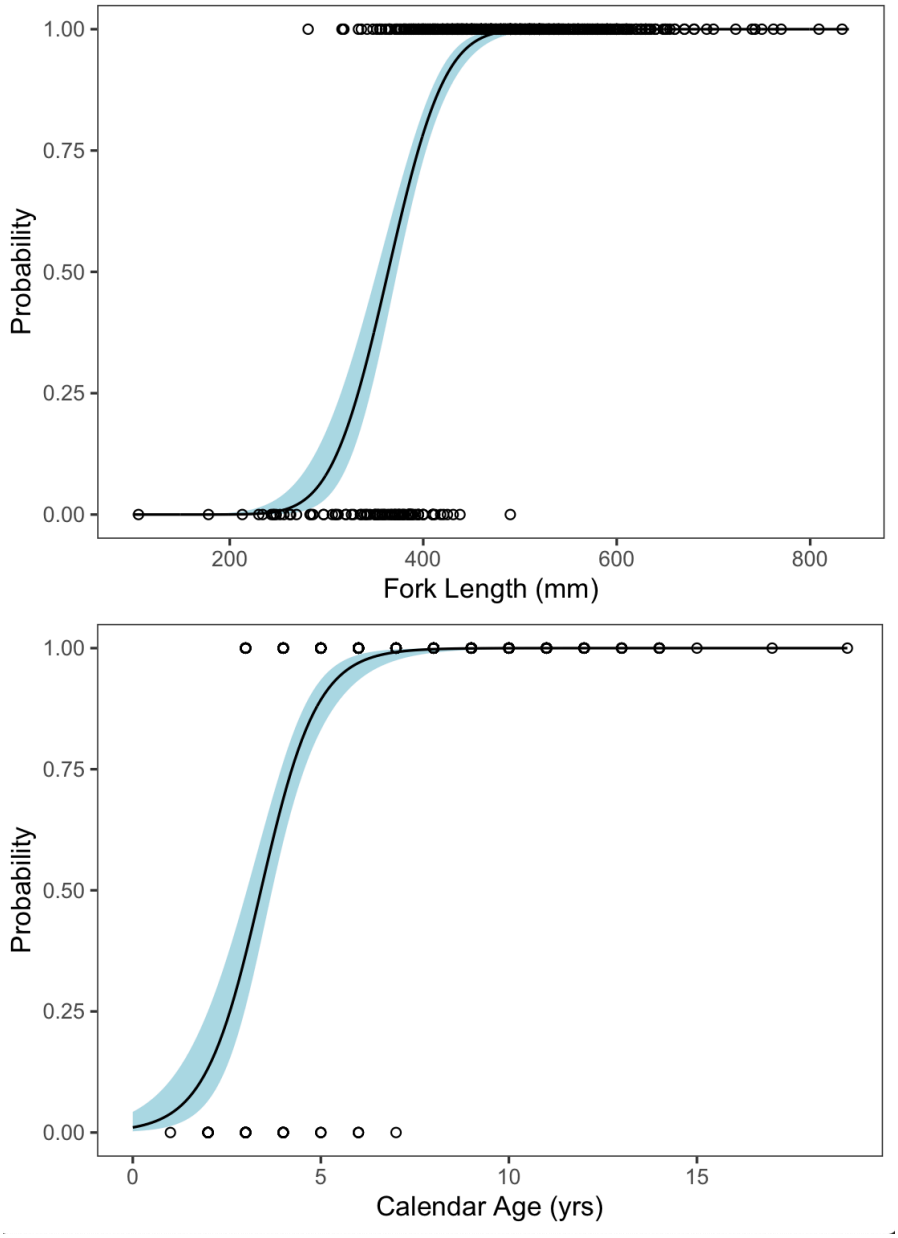


Figure 6. Observed and predicated length (top) and age (bottom) at maturity with 95% confidence intervals. The estimated size at 50% maturity under the best-fitting model (probit) was 363.7 mm FL, and the estimated age at 50% maturity under the best-fitting model (logit) was 3.4 years.

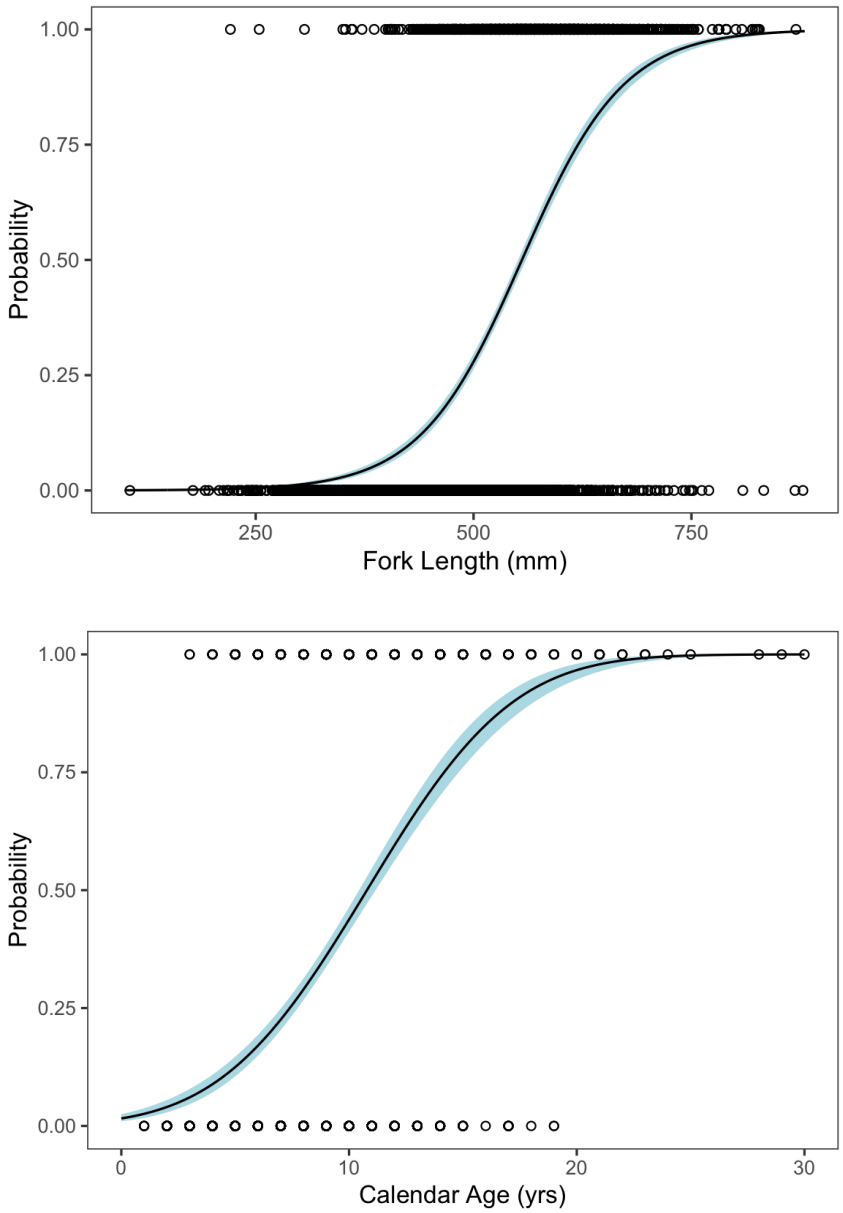


Figure 7. Observed and predicated length (top) and age (bottom) at transition with 95% confidence intervals. Estimated size at 50% male under the best-fitting model (logit) was 555.6 mm FL, and estimated age at 50% male under the best-fitting model (probit) was 10.8 years.

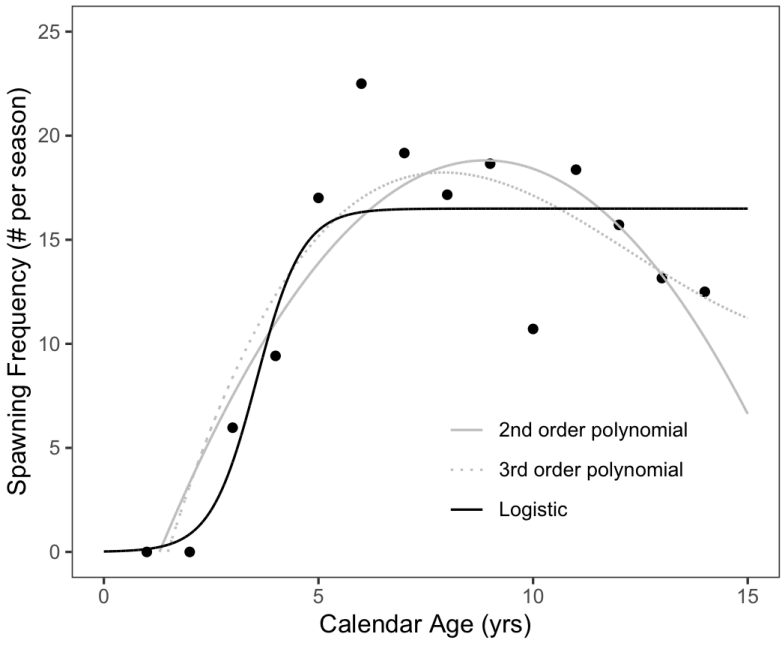


Figure 8. Estimated spawning frequency at age (filled circles) and the three best-fitting models for the base spawning season length of 100 days. The logistic provided the best fit (black line), followed by a second-order polynomial (grey solid line) which was a marginally better fit compared to the third order polynomial (dotted grey line). Ages 14 through 19 were pooled. Spawning frequency was estimated using all (mature and immature) females with available age information.