Mutton Snapper Reproduction

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Mutton Snapper reproduction

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Introduction:

Mutton snapper (*Lutjanus analis*) supports important recreational and commercial fisheries in the Caribbean and the Southeastern United States (primarily the Florida Keys and Southeast Florida). Mutton snapper have been reported to form large spawning aggregations at specific spawning sites (Claro et al., 2009), typically at the time of the full moon in March through July (Heyman and Kjerfve 2008; Feeley et al., 2018; Heidmann et al., 2021). However, peak spawning activity can vary even within a country, as in Cuba where peaks can occur between May and August depending on the location (SCRFA https://www.scrfa.org/aggregations/aggregating-species/mutton-snapper/). The aggregating spawning behavior of mutton snapper makes them vulnerable to overfishing and the IUCN has classified the species as "Near Threatened". Known spawning aggregation sites within the U.S. include Riley's Hump and the Dry Tortugas.

Mutton snapper exhibit spatial separation of adult and juvenile members of the local population. After a pelagic larval period of ca. 31 days, mutton snapper settle onto a suite of available habitats, including nearshore vegetated habitats such as seagrass beds < 10 m deep (Lindeman et al. 2000). Although data are limited, evidence suggests that mutton snapper undergo ontogenetic habitat shifts from shallow vegetated habitats to alternative structure, including the reef tract, in response to increased body size decreasing predation mortality in adult habitats (e.g., Dahlgren and Eggleston 2000).

Methods

Standardizing the reproductive data:

The analyses presented here are based only on females. The original mutton snapper reproductive database had 3,671 fish assigned as female and three types of indicators of ovarian development: 3,653 fish with a macroscopic reproductive phase, 632 fish with a histological reproductive phase, and 3,038 fish with gonad weight and total weight, needed to calculate the gonadosomatic index (GSI) as:

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

Histological analysis is considered the most accurate method to assess gonadal development, with reproductive phase assigned based on the most advanced gamete stage (MAG) and/or post-ovulatory follicles and atresia (Table 1). Macroscopic evaluation of ovaries is less precise than histological assignment but best practices to use this indicator have recently been developed (Table 2). Macroscopic analysis cannot identify immature fish or accurately distinguish between regenerating, developing, and regressing, and these phases are assigned as mature, undeveloped (MU). Because fully yolked oocytes are typically pale yellow and ~0.50 mm, they can be identified macroscopically and used as a phase indicator (YO – yolked oocytes). Ovaries assigned as YO can also have fresh POFs which would not be macroscopically visible. Also hydrated oocytes are typically ~1.0 mm and are easily identifiable macroscopically both before and after they are ovulated.

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The first step in our mutton snapper reproductive data QC process was to verify sex assignment and select for only females. All individuals selected for the present analyses were confirmed to be female either based on histological sex assignment or presence of female spawning indicators / reproductive phases. A total of 3,673 confirmed females had either a macroscopic or histological reproductive phase. We then evaluated where and when fish were sampled, as size and age at maturity is not invariant and is often affected by changes in temperature regimes or fishing mortality. The vast majority of the samples (99.8%) came from the Florida Keys or SE Florida and were collected over two time periods: (1) from 1998-2004 (fishery independent sampling (2) in 2007-2011 (fishery dependent sampling) and this is the data set analyzed here, including 3,655 females with a reproductive phase from the Florida Keys (n=997) or off Southeast Florida (n=2667) sampled from 1998 to 2011. Fishery independent sampling methods included Chevron traps, hook and line, and spearfishing. This data set was first described by Barbieri and Colvocoresses (2003), with 28 additional fish sampled in the Florida Keys in 2004.

A lack of standardized criteria and names for reproductive phases makes it difficult to conduct reproductive analyses on databases from multiple studies. Several webinars were held in 2022 to build on standardization presented in Lowerre-Barbieri et al. (2011) and Brown-Peterson et al. (2011) and form a best practices approach to reproductive data. The developed criteria were then tested in two stock assessments: SEDAR 72 for red snapper and here. We first evaluated the range of female macroscopic reproductive phase names and their corresponding numeric categories. There were 22 macroscopic phase names and 13 numeric categories. This level of detailed development cannot be accurately assigned macroscopically (i.e., subphases of early and late development). Fish with noninformative macroscopic reproductive phase names (i.e., no clear connection with development) and those assigned as “unidentified” were reassigned as DN (did not attempt or does not have). These phase names included: “intermediate size”, “Intermediate size but lacking milt” “Near maximum size”, “Unidentified” and “Presumably mature but of small size”. The remaining descriptive names were assigned to the following macroscopic reproductive phase names based on the SEDAR best practices template (i.e., mature undeveloped (MU), yolked (YO), actively spawning (AS), does not have (DN)). Histological phase names were similarly assessed by first looking at the range of names and removing those that were uninformative and without other corroborating data. Numeric categories for histological phases (1-4, 6 and 7) were consistently assigned to development although phase names varied. Thus, it was relatively easy to reassign numeric categories and standardize phase names to: 1=immature, 2=developing, 3=spawning capable, 4=actively spawning, 5=regressing, and 6=regenerating and then standardize the names (Table 1).

To determine if consistent histological criteria were used to assign histological numeric categories, we reviewed the phase names and MAG for discrepancies. There were five discrepancies, all in the spawning capable reproductive phase. These were reassigned to the appropriate histological reproductive phase (four females with hydrated oocytes were assigned as active spawners and one female which only had cortical alveolar oocytes was assigned as developing). Because phase assignment is always somewhat subjective and affected by experience, we retained only females which had a reproductive phase and GSI (n=3,031). This resulted in all retained females having two indicators of ovarian development. Lastly, we assessed the mean, median, and range of GSI by histologically assigned

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reproductive phase to identify outliers and expected ranges. One outlier (GSI > 1) was identified in regressing females. Given its macroscopic assignment as YO and a MAG of V (vitellogenic or yolked), its histological reproductive phase was changed to spawning capable.

Analysis

The clean data set had a total of 876 fish sampled in the Keys (1998-2004, n=171; 2007-2011, n=705) and 2,155 fish sampled in the SE Florida (1998-2004, n=159; 2007-2011, n=1996). There is no definitive histological indicator to distinguish immature from mature regenerating females, which both have only PG oocytes. Criteria used for maturity assignment are a major source of uncertainty for estimating maturity at age. We considered using gonadosomatic index (GSI) to help distinguish between resting and immature females.

Spawning seasonality

Spawning seasonality was based on ovarian development, with the macroscopic phases “YO” and “AS”, and histologic phases “spawning capable” and “actively spawning” indicative of the spawning season. Typically, peak spawning is based on months with a high percentage (~75%) of spawning capable females (Lowerre-Barbieri et al. 2009).

Maturity

Fitting a logistic curve to sex-specific maturity data distributed by size or age is the traditional method of estimating size and age at 50% sexual maturity. However, the accuracy of the resulting estimate will be affected by the spatial distribution of sampling relative to that of nursery and adult habitat, the time period over which samples are collected, and the method used to categorize fish as mature or immature (Hunter and Macewicz 2003; Lowerre-Barbieri et al., 2011). Here we use binomial generalized linear models (GLMs) to model maturity at age and length, with different link functions (logit, probit, cloglog and cauchit) specified, and the best model chosen via corrected Akaike Information Criterion (AICc). Models were fitted in R and model comparison was performed using the R package ‘MuMIn’. Estimated parameters were the intercept and slope. For the logit link function, the binomial GLM model parameters, intercept and slope, can readily be translated to fit the logistic function of the form Maturity[y] = 1/(1+exp(-a*(y-b))). The inflection point (*b*; age or length at 50% maturity) is calculated by dividing the negative value of model intercept by slope, and steepness (*a*) is the model slope. The standard error for *b* was calculated using the propagation of errors formula SE(*b*) = |*b*| * √((SE(intercept)/intercept)^2 + (SE(slope)/slope)^2).

Several approaches were explored for time period selection and maturity indicators. The traditional approach is to use histologically assigned reproductive phases and filter for dates within the core spawning season to decrease the number of regenerating females that might be misidentified as immature (Hunter and Macewicz 2003). However, this approach appears to work best for fishes with constricted spawning seasons. In contrast mutton snapper, like red snapper, have extended spawning seasons and regenerating females occur within the spawning season and even peak spawning months.

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To increase sample sizes for species with these patterns we developed a method that drops the seasonal filter and uses a conservative approach to assigning maturity based on reproductive phases (Lowerre-Barbieri et al., 2022). With this method only fish assigned as immature histologically are used and mature fish are represented by those either confirmed as mature because they are active spawners or when needed including those which have yolked oocytes and are spawning capable. Here we used histologically assigned immature (IM), macroscopically and histologically assigned ovaries with yolked oocytes (YO and spawning capable) and active spawners.

Results / Discussion:

Spawning season

Macroscopic phase data was used to evaluate spawning seasonality, given the large sample sizes (and very small histological sample sizes) and the high accuracy of macroscopic staging for yolked and active spawners. Females with yolked oocytes (YO macroscopic phase) occurred throughout the year in the Florida Keys and in most months in SE Florida, although in much lower numbers (Fig. 1). Active spawners first occurred in April (n=2) and were last sampled in September (n=1, none were sampled in August; Fig. 2). Based on elevated GSIs and proportion of spawning capable females (YO and AS), we consider April through July to be the core spawning season (Fig. 1).

The data set has a low frequency of spawning indicators, especially in SE Florida. Most females macroscopically staged were “UN” (undeveloped, 89%), 10% were YO (yolked oocytes) and only 1% were active spawners (AS). Histological analysis confirmed very low proportions of active and spawning capable females, as well as an extended time period over which developing, regressing, and regenerating females occurred (Fig. 3). The peak proportion of spawning capable females (YO and AS) occurred in the Florida Keys in May and June (Fig. 2), but even in these months it was only ~60% in the Keys and never surpassed 10% in SE Florida. Mutton snapper are reported to aggregate to spawn, at least at Riley’s Hump, where fish were present from April to August for ~one week at a time. Some fish returned 2-3 times within a spawning season (Feeley et al., 2018). Typically, when a species moves to a location specifically to spawn the proportion of active spawners is much higher. For example, at a spotted seatrout aggregation site, the proportion of females that were actively spawning was 91% (Lowerre-Barbieri et al., 2009). However, like spotted seatrout, mutton snapper may not all exhibit the same reproductive strategy in terms of spawning site selection.

Additional research is needed to better understand mutton snapper reproduction in the US. It is important to note that all reproductive data used here were more than 10 years old. Because reproductive timing in spring and summer-spawning fish is tightly coupled to temperature (Lowerre-Barbieri et al, 2011) spawning seasonality may have changed with climate change. In addition, the data are suggestive of potential migration through SE Florida to Keys spawning grounds and possibly even a second spawning season. Understanding these processes will be critical to estimating annual fecundity in this species.

Maturity

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We found significant overlap between GSI of immature and mature, non-spawning females, as assigned via histology (Fig. 5), indicating that GSI cannot be used to differentiate between immature and mature females. We therefore only included immature individuals assigned via histology, and mature fish designated as spawning or spawning capable either using histology or macroscopic staging in the maturity analysis. We note that, of the 11 individuals assigned as immature using histology, ten were assigned as regenerating and only one as immature based on macroscopic staging.

Of the fish histologically assigned as immature, all came from fishery-independent sampling and from period 1 (sampled prior to 2003), suggesting that fishery independent (FI) samples are needed to supplement FD samples which are above the minimum size limit (Fig. 4). The smallest mature female mutton snapper (YO, SC or AS reproductive phases) was 405 mm natural total length (TL) and the largest immature female observed via histological staging was 425 mm TL (Tables 3 and 6; Fig. 6). The youngest mature female mutton snapper was 3 years old, and the oldest immature female observed via histological staging was 4 years (Tables 4 and 6, Fig. 7). The logit model was within 2 delta AICc values of the best model for all model runs, and thus we report model parameters for the logit model here. Length and age at 50% maturity were estimated as 422 mm TL and 3.5 years, respectively, when only spawning capable and actively spawning females were included as mature and no temporal filter was applied. When we included all non-regenerating females with histological data and sampled during the spawning season, we obtained smaller estimates of size and age at maturity, 387 mm TL and 2.4 years, respectively (Table 5, Figs 6 and 7).

Comparison to previous maturity estimates:

The method of assessing maturity developed for species with extended spawning seasons (Lowerre-Barbieri et al., 2022) and applied here uses only histologically assigned immature females and yolked and actively spawning females (both macroscopically and histological staged). As in previous analyses regenerating females were removed. In addition, here we removed developing females as well. However, the resulting sample size was 274, greatly increased by not filtering for core spawning months. The resulting inflection point estimates using this approach (L50=422 mm TL and A50=3.5 years) are similar to those from the first benchmark assessment (L50=402 mm TL, A50=3.71 years; SEDAR 15A 2008), but larger than those from the update assessment (L50=398 mm TL, A50=2.85 years; SEDAR 15AU 2015).

In SEDAR 15A 2008, female reproductive phases were assigned via histology (n=310) from 999 fishery independent samples (Barbieri and Colvocoresses 2003). After filtering for core spawning months (April through June) and removing regenerating females only 39 samples were left for estimating maturity parameters. For the update assessment completed in 2015 (SEDAR 15AU 2015), additional maturity data included fishery dependent data collected as part of a cooperative research study (Cody and Poholek 2011). Available data had reproductive phases assigned via macroscopic evaluation. Filtering for core spawning months and removing regenerating females resulted in 192 samples to update the maturity-at-age relationship and 221 samples to update the maturity-at-length relationship. However, only 38 were based on histological analysis. Because immature fish cannot be accurately assigned with macroscopic staging, this presumably affected the L50 and A50 estimates.

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References

Barbieri, L.R., and J.A. Colvocoresses. 2003. Southeast Florida reef fish abundance and biology. Five-year performance report for Florida grant F-73 to the U.S. Fish and Wildlife Service, Dept. of Interior, 120 pp.

Brown-Peterson, N., Wyanski, D., Saborido-Rey, F., Macewicz, B., and Lowerre-Barbieri, S. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics. Management, and Ecosystem Science [online serial] 3, 52-70.

Claro, R., Sadovy de Mitcheson, Y., Lindeman, K.C., Garcia-Cagide, A.R., 2009. Historical analysis of Cuban commercial fishing effort and the effects of management interventions on important reef fishes from 1960–2005. Fish. Res. 99, 7–16.

Cody, R., and Poholek, A. (2011). Reproductive Biology and Ecology of Important Shallow-water Snapper Species in South Florida and the Florida Keys. Cooperative Research Grant Final Report. Grant Number: NA09NMF4540139.

Dahlgren, C.P., and Eggleston, D.B. (2000). Ecological Processes Underlying Ontogenetic Habitat Shifts in a Coral Reef Fish. Ecology 81, 2227.

Heyman WD, Kjerfve B. 2008. Characterization of transient multi-species reef fish spawning aggregations at Gladden Spit. Belize Bull Mar Sci. 83(3):531–51.

Hunter, J. R., and B. J. Macewicz. 2003. Improving the accuracy and precision of reproductive information used in fisheries. Pages 57–68 in O. S. Kjesbu, J. R. Hunter, and P. R. Witthames, editors. Report of the working group on modern approaches to assess maturity and fecundity of warm- and cold-water fish and squids. Institute of Marine Research, Bergen, Norway.

Feeley, M.W., Morley, D., Acosta, A., Barbera, P., Hunt, J., Switzer, T., and Burton, M. (2018). Spawning migration movements of Mutton Snapper in Tortugas, Florida: Spatial dynamics within a marine reserve network. Fisheries Research 204, 209-223.

Heidmann, S.L., Jossart, J., Kimble, M., and Nemeth, R.S. (2021). Home range characteristics and diel patterns in space use of mutton snapper, Lutjanus analis, in St. Thomas, US Virgin Islands. Animal Biotelemetry 9.

Lindeman, K.C., Pugliese, R., Waugh, G.T., and Ault, J.S. (2000). Developmental patterns within a multispecies reef fishery: management applications for essential fish habitats and protected areas. BullMarSci 66, 929-956.

Lowerre-Barbieri, S.K., Henderson, N., Llopiz, J., Walters, S., Bickford, J., and Muller, R. (2009). Defining a spawning population (Spotted Seatrout Cynoscion nebulosus) over temporal, spatial, and demographic scales. Marine Ecology Progress Series 394, 231-245.

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Lowerre-Barbieri, S., Ganias, K., Saborido-Rey, F., Murua, H., and Hunter, J. (2011). Reproductive timing in marine fishes: variability, temporal scales, and methods. Marine and Coastal Fisheries: Dynamics. Management, and Ecosystem Science [online serial] 3, 71-91.

Lowerre-Barbieri, S., C. Friess, N. Brown-Peterson, H. Moncrief-Cox, and B. Barnett. 2022. Best practices for standardized reproductive data and methodology to estimate reproductive parameters for Red Snapper in the Gulf of Mexico. SEDAR74-DW-36. SEDAR, North Charleston, SC. 43 pp.

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Tables and Figures

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.

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Table 2. Slightly modified table of best practices field names, description and acceptable values for female reproductive data. We renamed the developing (DV) macroscopic reproductive phase, "yolked" (YO) to distinguish it from the developing histological phase which represents those females which are developing but have few or no yolked oocytes.

Field names	**Description**	**Acceptable Values**
Gonad_Observed	Observed in the field (macro assessment, gonad weight)	Y – Yes N – No
Histo_Taken	Tissue - histologically processed	Y – Yes N – No
Macro_Sex	Sex identified by field sampler based on macroscopic appearance of gonad	F – Female M – Male T – Transitional U – Unknown Sex
Macro_Repro_Phase	Maturity based on macroscopic evaluation of reproductive tissue	IM – Immature UN – Undeveloped (immature, spent/regressing, resting/ regenerating) YO – Clearly identifiable yolked oocytes AS – Active Spawner (hydrated oocytes; milt) DN – Did Not Attempt or does not have
Histo_Sex	Sex assigned after histological reading of gonad tissue	F – Female M – Male T – Transitional U – Unavailable/Lost N – Not Gonad Tissue
Historic_Data	Any data not recorded following Brown-Peterson et al. (2011)	Y – Yes N – No
Histo_Repro_Phase	Standardized terminology that includes both males and females. Reference documents (Brown-Peterson et al. 2011, Table 2 and 3; see also Lowerre-Barbieri et al. 2009 Table 1)	IM – Immature DV – Developing SC – Spawning Capable AS – Active Spawner RG – Regressing RN – Regenerating TR – Transitional
Most_Advanced_ Gamete_Stage	Stage must occur in $\geq$ 5% of the slide to be considered "most advanced". Scan of the entire slide: 4x on female tissue.	PG – Primary Growth CA – Cortical Alveolar V1 – Primary Vitellogenesis V2 – Secondary Vitellogenesis V3 – Tertiary Vitellogenesis LC – Lipid Coalescence YC – Yolk Coalescence GM – Germinal Vesicle Migration GB – Germinal Vesicle Breakdown HO – Hydration/Ovulation If Historic data: VT – Vitellogenesis OM – Oocyte Maturation
POF	Spawning frequency phase indicator	N - None C – Newly Collapsed R – Recent (can be used for spawning frequency)

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		B - Both Newly Collapsed and Recent POFs present If Historic data: P – Present, unknown age
Atretic yolked oocytes	Used to identify regressing females	A-Alpha atresia of yolked oocytes
Histological_ Indicator_1	Other structures found within the histological section that support Histo_Repro_Phase classifications, especially in the case of immature vs regenerating specimens	MB – Muscle Bundle AB – Alpha/Beta atresia of yolked oocytes GD – Gamma/Delta atresia AU - Atresia of Unyolked oocytes BV – Blood Vessels evident throughout TN – Thin Ovary Wall TK – Thick Ovary Wall

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Table 3. Predicted and observed maturity at natural total length from binomial model fit with logit link for the model that included all sampling months and spawning capable or actively spawning females assigned through histology and macroscopic staging in the mature group. Total N_{obs} = 274.

Length bin midpoint	N	N_{mat}	Observed Prop Mature	Predicted Prop Mature
225	1	0	0.0000	0.0000
275	1	0	0.0000	0.0000
325	17	0	0.0000	0.0000
375	30	0	0.0000	0.0028
425	19	10	0.5263	0.6046
475	13	13	1.0000	0.9988
525	12	12	1.0000	1.0000
575	10	10	1.0000	1.0000
625	29	29	1.0000	1.0000
675	36	36	1.0000	1.0000
725	51	51	1.0000	1.0000
775	38	38	1.0000	1.0000
825	16	16	1.0000	1.0000
875	1	1	1.0000	1.0000

Table 4. Predicted and observed age at maturity from binomial model fit with logit link for the model that included all sampling months and spawning capable or actively spawning females assigned through histology and macroscopic staging in the mature group. Total N_{obs} = 240.

Age	N	N_{mat}	Observed Prop Mature	Predicted Prop Mature
1	3	0	0.0000	0.0001
2	28	0	0.0000	0.0039
3	26	4	0.1538	0.1426
4	14	12	0.8571	0.8752
5	20	20	1.0000	0.9966
6	34	34	1.0000	0.9999
7	17	17	1.0000	1.0000
8	25	25	1.0000	1.0000
9	14	14	1.0000	1.0000
10-29	59	59	1.0000	1.0000

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Table 5. Parameter estimates for mutton snapper maturity binomial generalized linear models with logit link function. Models were fitted to 1) only immature and spawning (as indicated by histo phases 3 & 4 and macro phases YO and AS) individuals without season filter and 2) immature and all non-regenerating (histo only) females filtered for individuals sampled during the spawning season (April – July). Only females assigned as immature via histology were included in all models. SS = Spawning season; HP = histophases; MP = macrophases. Note that intercept and slope are the GLM model parameters on the logit scale, but slope is also the steepness of the logistic model of the form p = 1/(1+exp(-a*(x-b))), and L50 is the inflection point of the logistic model, calculated as -intercept/slope.

Response	Season	Mat_phases	N	Parameter	Estimate	St.Error
TL	All Year	Histo & Macro SC & AS	274	Intercept	-53.021	17.497
				Slope	0.126	0.042
				L50	421.6	197.8
	Spawning Season	Only Histo except Regenerating	74	Intercept	-44.681	18.733
				Slope	0.115	0.048
				L50	387.4	229.2
Age	All Year	Histo & Macro SC & AS	240	Intercept	-13.021	2.809
				Slope	3.742	0.835
				A50	3.479	1.08
	Spawning Season	Only Histo except Regenerating	68	Intercept	-6.054	2.105
				Slope	2.535	0.787
				A50	2.389	1.11

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Table 6. Sample sizes, minimum and maximum observed lengths and ages of immature and mature females for the different methods of data subsetting considered. Immature were always histo only samples. No estimates could be produced for the "Spawning season, Histo & Macro SC & AS" method, as the lengths and ages of mature and immature individuals did not overlap. RN = Regenerating, SC = Spawning capable, AS = Actively spawning.

Response	Season	Mat_phases	Maturity	N	Min_obs	Max_obs
TL	All Year	Histo & Macro SC & AS	Immature	58	227	425
			Mature	216	405	863
	Spawning Season	Histo & Macro SC & AS	Immature	11	325	396
			Mature	197	405	850
	Spawning Season	Only Histo except Regenerating	Immature	11	325	396
			Mature	63	375	815
Age	All Year	Histo & Macro SC & AS	Immature	55	1	4
			Mature	185	3	29
	Spawning Season	Histo & Macro SC & AS	Immature	10	2	4
			Mature	167	3	29
	Spawning Season	Only Histo except Regenerating	Immature	10	2	4
			Mature	58	2	17

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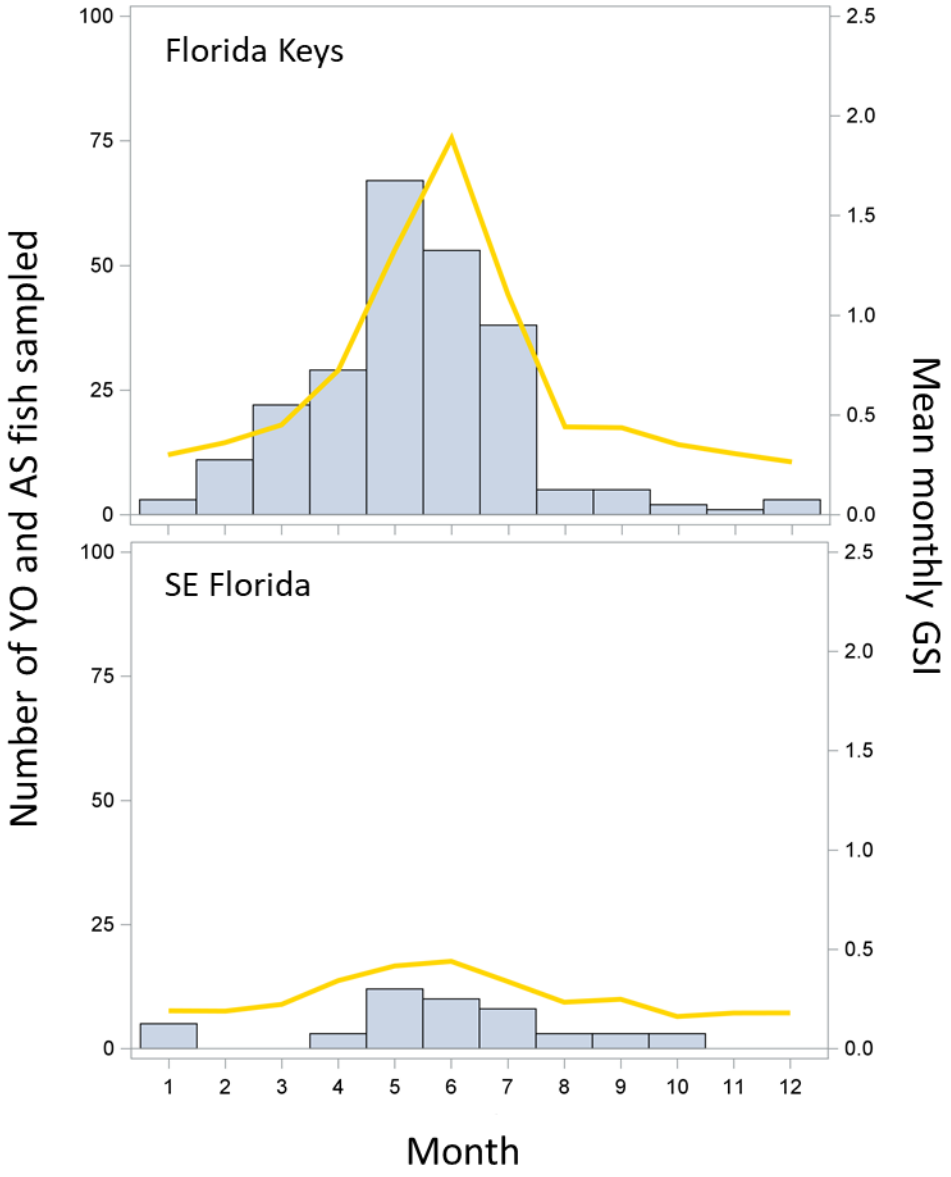

Figure 1. Females macroscopically staged as having yolked oocytes (macroscopic reproductive phase "YO") occurred in all months in the Florida Keys and most months in SE Florida. The number of YO females peaked in May, with GSI of all females peaking in June. GSI and number of YO females was quite low in SE Florida throughout the year.

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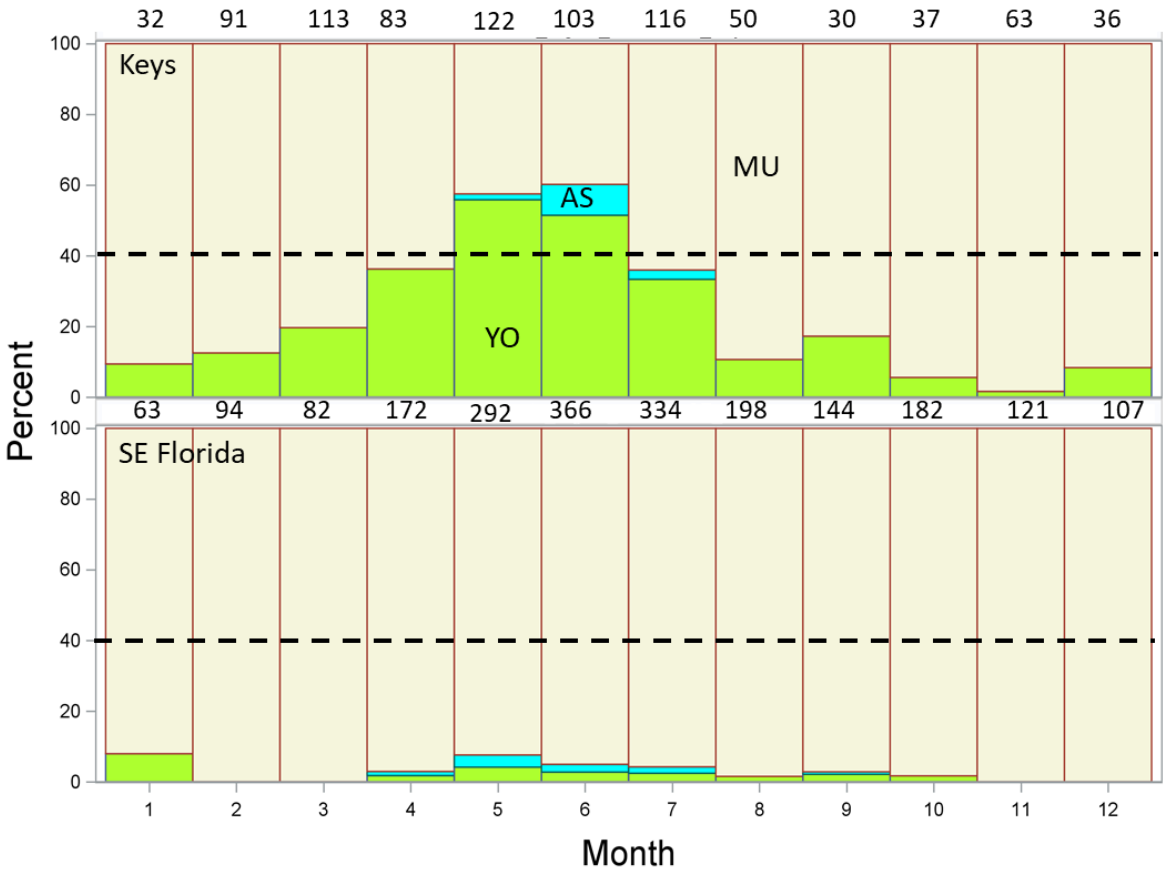

Figure 2. Monthly proportion of macroscopic phases (YO=yolked, AS=active spawner, MU=mature undeveloped). Peak spawning occurred in May and June when the proportion of spawning capable females (YO and AS) was > 40%. The reference line indicates 40%.

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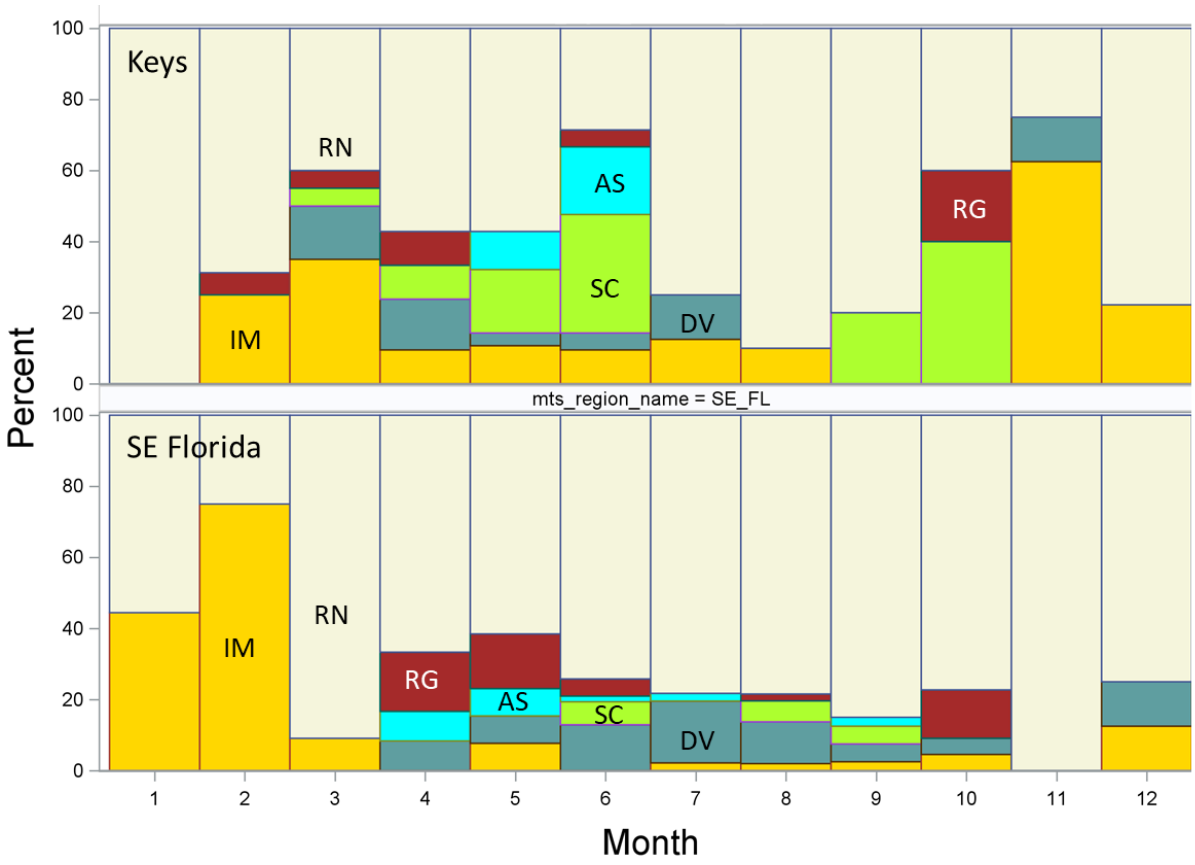

Figure 3. Frequency plot of monthly histological reproductive phases by study area (Keys: n=156; SE Florida, n=294). IM = Immature, DV = Developing, SC = Spawning capable, AS = Active Spawner RG = Regressing, RN=regenerating.

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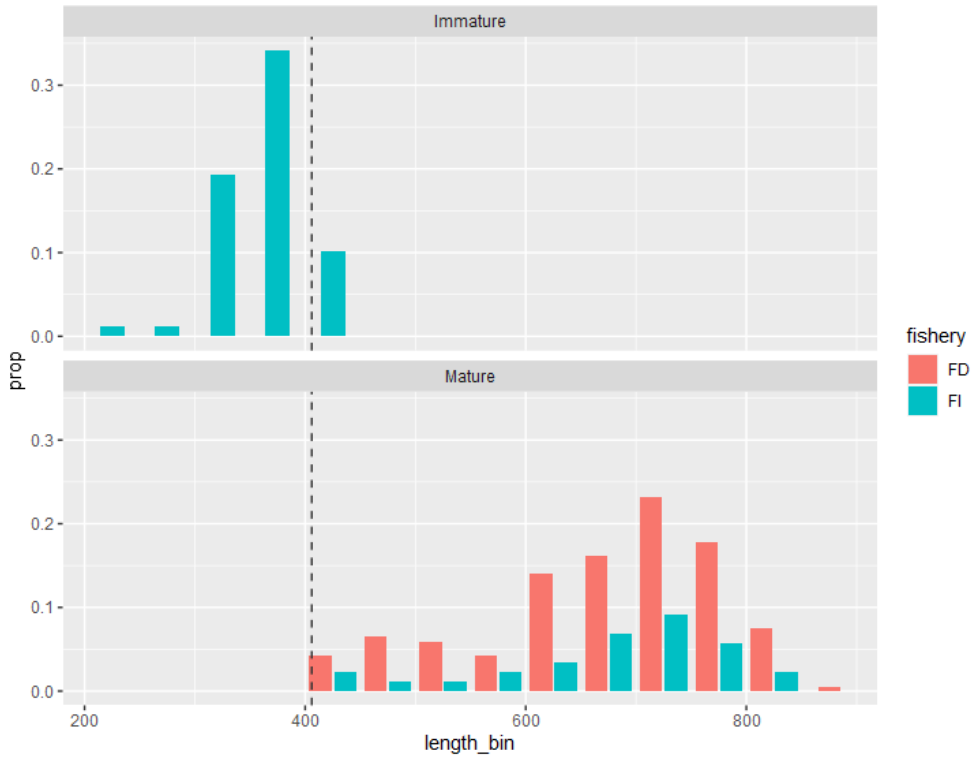

Figure 4. Length distribution (shown as proportions by fishery; FI = fishery-independent, FD = fishery-dependent) for the 274 females used in the recommended maturity-at-length model (All year, Histo and Macro spawning capable and actively spawning) for natural total length. All immature individuals came from FI sampling, and most of the FI samples were immature. The dotted grey line is the minimum size limit of 16 inches.

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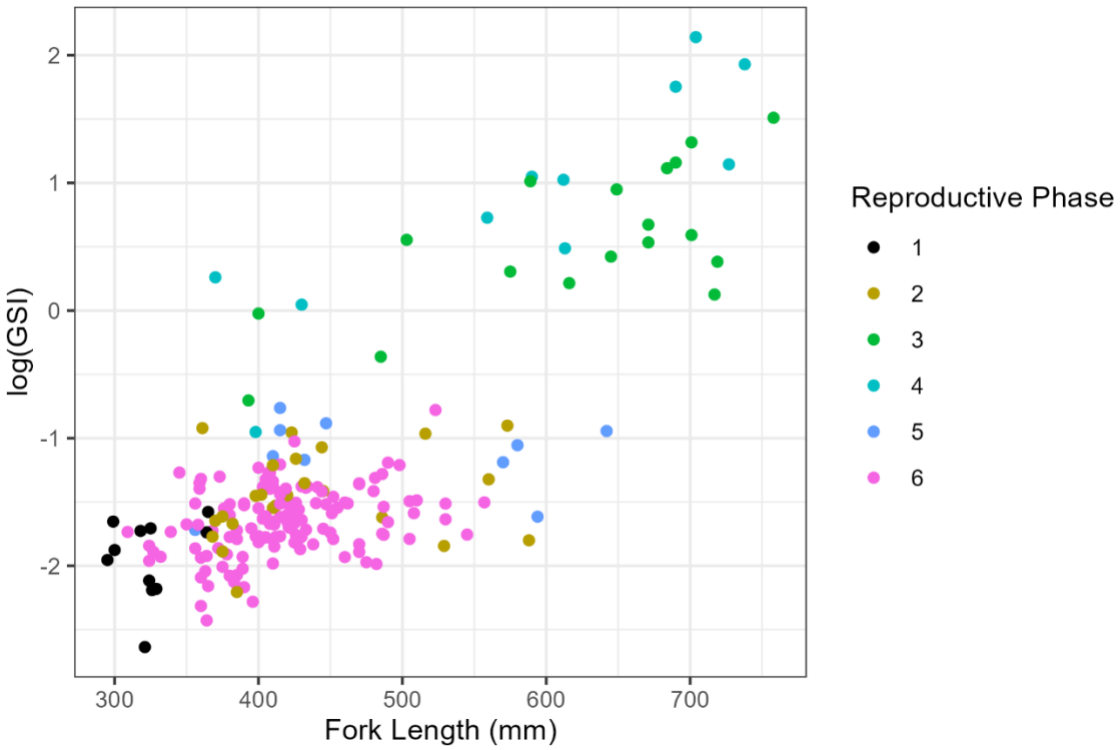

Figure 5. GSI in log space as a function of fork length for mutton snapper samples assessed via histology (n = 213), showing GSI cannot be used to distinguish immature (Reproductive Phase 1) individuals from mature, non-spawning individuals.

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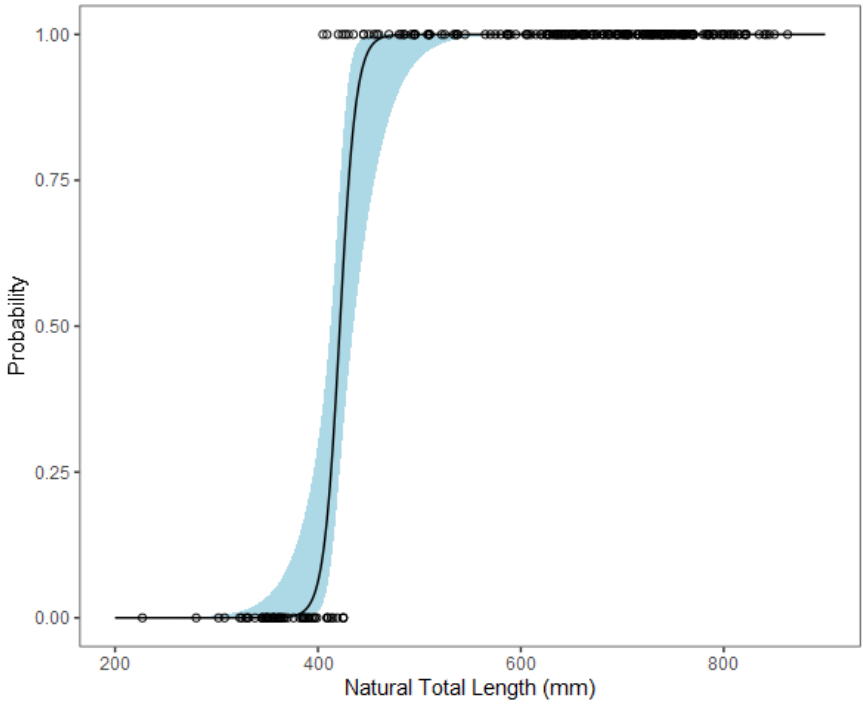

Figure 6. Observed (n = 274) and predicated fork length at maturity with 95% confidence intervals for the model that included all sampling months and spawning capable or actively spawning females assigned through histology and macroscopic staging in the mature group. The estimated size at 50% maturity for this model was 422 mm natural total length.

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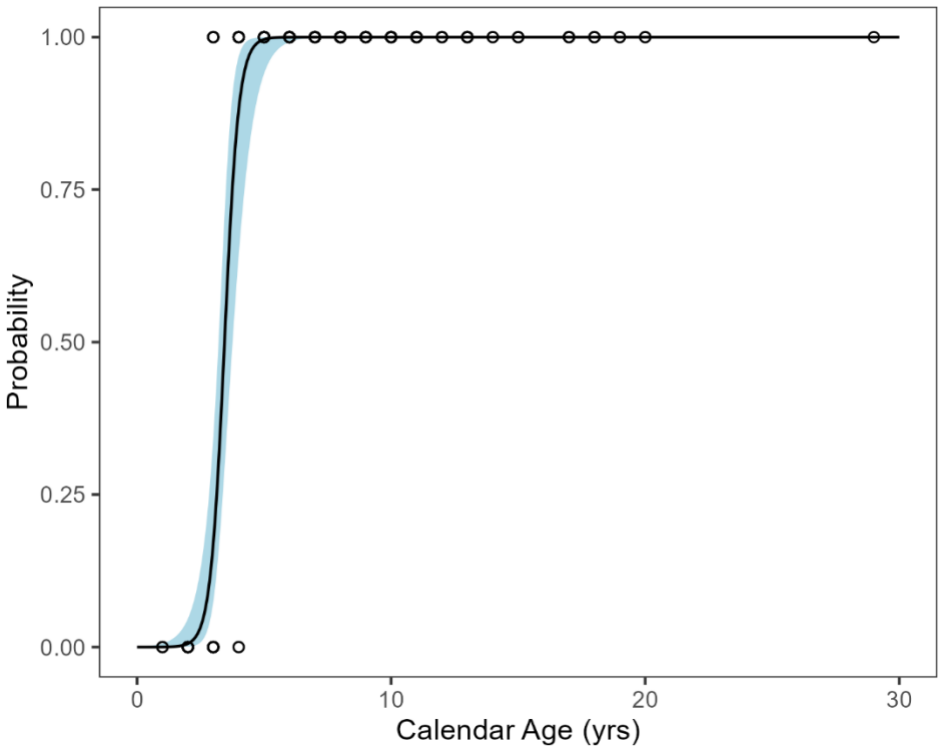

Figure 7. Observed (n = 240) and predicated age at maturity with 95% confidence intervals for the model that included all sampling months and spawning capable or actively spawning females assigned through histology and macroscopic staging in the mature group. The estimated age at 50% maturity for this model was 3.5 years.

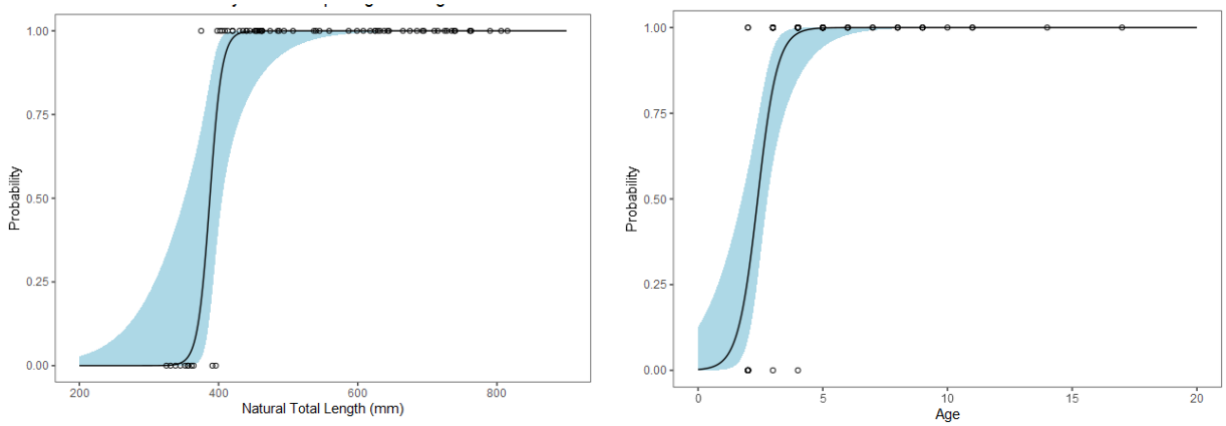

Figure 8. Results for the model that included only histo data except regenerating for the main spawning season months (April – July). See table 6 for model parameters and sample sizes.